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During the year 1918 the following persons have been connected with or have done work under the auspices of the Otho S. A. Sprague Memorial Institute:

Director: H. Gideon Wells.

Members: R. T. Woodyatt, Lydia M. DeWitt, Samuel Amberg, Henry F. Helmholz, Maud Slye, Karl K. Koessler, E. J. Witzemann.

Associate Members: Russell M. Wilder, W. D. Sansum, Julian H. Lewis, M. T. Hanke.

Voluntary Investigators: Harriet F. Holmes, J. P. Simonds, Noriyoshi Kimura, George T. Caldwell, Sukemasa Ogata.

Assistants: Mary E. Maver, Carol Beeler, Edith Farrar, H. L. Huber, J. O. Balcar, S. M. Cadwell, Binzi Suyenaga, Henry C. Sweaney, Gladys Leavell, Maria B. Maver, Morris Kharasch, Friedrich Lommen.

Fellows: L. W. Sauer, Peter Mattill, Katherine Mayer, Verne E. Eastman, Herbert O. Lussky, W. B. McClure.

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THE WORK OF THE OTHO S. A. SPRAGUE MEMORIAL INSTITUTE DURING THE WAR

Immediately after America entered the war the Sprague Institute formally offered its services to the Government through the National Research Council, which services were accepted by the chairman of the committee on Medicine and Hygiene, Dr. V. C. Vaughan. The request was made that as far as possible the Institute be held together, with the research work continuing along the same lines as previously, in order to be ready whenever the Government should submit special problems to us, and this plan was followed for a time. As, however, the National Research Council did not soon become organized in such a way as to submit much work to the Institute, a few of the members found it desirable to undertake work directly for the army or the Government. The work of the Institute, therefore, consisted of that done in the laboratories of the Institute and that done by its members in various services of the army, and may best be indicated by describing the services of the various members and laboratories separately.

The Director, Dr. H. Gideon Wells, was appointed a member of the Medicine and Hygiene Committee of the National Research Council on January 25, 1917. In July, 1917, Dr. Wells was given leave of absence by the Trustees to accept a commission as Major in the American Red Cross, to serve as a member of the Commission to Roumania. In this capacity he was absent five months, during which time he travelled via Japan, Siberia and Russia to Roumania and helped organize the defense of the country against typhus and other epidemic diseases. A full report of this work was published in collaboration with Major R. G. Perkins of Cleveland in the *Journal of the American Medical Association,* March 16, 1918.

On returning to America he engaged in active propaganda work, describing the part played by Roumania in the war, its accomplishments, its needs and its rights, and also the work of the Red Cross in that country. On April 24, 1918, he was appointed a member of the Medical Advisory Council of the Gas Defense Section of the Chemical Warfare Service, U. S. A. In this capacity he attended regular meetings of the Council in Washington to advise on the problems of this branch of the service, and to plan for work to be done by the Sprague Institute in this field, discussed elsewhere in this report. In June, 1918, he was appointed Chairman of the Committee on Industrial Poisonings among Munitions Workers under the National Research Council, and helped to organize research work in this subject in the United States. To this committee, which had members representing the Ordnance Department of the Army, the Bureau of Labor and the Hygienic

Laboratory of the Public Health Service, were submitted problems and questions arising in connection with the poisoning of persons engaged in handling munitions, especially high explosives, or concerning the possibility of poisoning from compounds the manufacture of which was contemplated. The research work was carried on in several laboratories in this country, including the Sprague Institute, up to the time of the armistice. To further this work the Sprague Institute provided three Fellowships for field workers who studied the conditions in the munitions plants and investigated the effects of the chemicals on the employees. The appointees were Mrs. Isabelle Fogo, who worked at the Du Pont plant in Haskell, N. J.; Richard W. Te Linde, at Penniman, Va., and Norton Eversoll at Barksdale, Wis. Their observations will be included in the final report on this work.

In October, 1918, the American Red Cross organized a Commission to the Balkan States, and Dr. Wells was asked to go with this commission as Director of Medical Affairs. Leave of absence was granted by the Trustees for this purpose, and Dr. Wells left Chicago October 25 on this assignment. He reached Paris on November 26, and undertook the task of organizing the medical relief work in those Balkan States where such work had not already been begun. The armistice having made entrance to Roumania possible, the American Red Cross War Council decided to send a separate commission to this, the largest of the Balkan States, and appointed Dr. Wells commissioner to Roumania with the rank of Lieutenant-Colonel. A unit of approximately sixty was organized, a 2,500 ton ship was secured from the Roumanian Government, loaded with supplies and equipment, and with the personnel of the Commission to Roumania, sailed from Toulon on February 8, 1918. Going by way of the Dardenelles the party reached Constanza, the Black Sea port of Roumania, on February 18, but this port could not be used because the bridge across the Danube had been blown up, and a wait of several days was necessary until an ice jam in the Danube River broke up. The port of Galatz on the Danube was reached February 25, from which port supplies and personnel were distributed over Roumania. Headquarters were established at Bucharest, from which the work in Roumania was administered. Cooperation was established with the Interallied Food Administration and the American Relief Administration, which also had their headquarters in Bucharest, so that all the relief work in Roumania was coordinated. On June 5, the work having then been fully organized and in full swing for some time, Dr. Wells was given permission to return to the United States to resume his work with the Sprague Institute and the University of Chicago, his place as head of the Roumanian Commission being taken by Lieut.Col. Henry W. Anderson, Commissioner to the Balkan States. He arrived in Chicago June 24, 1919.

In the absence of Dr. Wells from the country in 1918-1919 Dr. Ludvig Hektoen served as acting director of the Institute.

The laboratories at the University of Chicago carried on their regular work during the war, but at reduced speed to permit of the taking up of whatever problems were submitted to them by the Government. The first problem was an investigation of a food recommended as an emergency ration for the army, and submitted to us for an opinion by the National Research Council. Analyses were made by Drs. George T. Caldwell and E. F. Hirsch, the results indicating that this particular food has no advantages over other non-patented foods of similar composition and lower prices.

In the spring of 1918 there was a severe epidemic of measles in many of the army camps, often complicated by a virulent pleurisy with high mortality. At the resquest of the surgeon-general's office, the director visited some of these encampments and made arrangements to have investigated certain of the problems presented. This work was carried out by Dr. Lydia M. DeWitt and Dr. Julian H. Lewis. Later a special commission was formed to work on this subject and the results obtained in the Sprague Institute were transmitted to this commission and the work dropped for other problems.

Some of the features of the investigation on the toxicity of war explosives were carried out by Drs. Lewis and Hanke, especially with reference to urine tests. Their results will be included in a final report on work with these explosives.

Dr. Julian H. Lewis was commissioned a First Lieutenant, M. R. C., November 10, 1917, but in order to permit him to continue investigative work for the Government in our laboratories, he was not called into active service. In addition to carrying on the investigations on empyema and the toxicity of war explosives, previously mentioned, he conducted experiments on the intravenous injection of antitoxic and other foreign sera at slow rates to avoid danger of anaphylactic shock. Also on the possibilities of long continued intravenous injections of ethylhydrocuprein (optochin) in the treatment of pneumococcus infections. Both these investigations were made with particular reference to war medicine, and have been published as follows: "Slow Intravenous Injection of Antiserum to Prevent Acute Anaphylactic Shock," *Jour. A. M. A.*, February 1, 1919. "Effect of Continuous Intravenous Injections of Ethylhydrocuprein," *Archives Int. Med.*, November, 1918.

In the summer of 1918 Miss Slye began raising mice for use by the army laboratories. The early termination of the war prevented the production of many mice for this purpose, but by the time of the armistice a stock of breeders had been developed from which large numbers of mice could have been obtained if it had been necessary.

At the Laboratory for Clinical Research at Rush Medical College, work was also carried on with the toxic war explosives. The Director,

Dr. R. T. Woodyatt, during the six months following our entry into the war, remained in charge of the laboratory in compliance with the request of the director and trustees of the Sprague Institute that the organization be kept running and available to the Government for assignments of war research problems. No assignments being made at first, work was undertaken on experimental therapy of phosgene gas poisoning in dogs by intravenous administrations of salts, alkali, sugar and oxygen gas, with no practical results, and on the treatment of surgical shock. The animal experiments on shock were made in collaboration with Prof. Joseph Erlanger of Washington University, in St. Louis, and the results published. (Erlanger and Woodyatt: Continued Intravenous Injection of Glucose in Shock, *Jour. Amer. Med. Assn.*, Oct. 27, 1917). Dr. Woodyatt was commissioned as Major, M. C., U. S. Army, February 6, 1918. His first assignment was to U. S. A. Base Hospital, Camp Cody, N. M., March 20. He reported for duty March 29, 1918, and served as Assistant Chief of Medical Service for three weeks, thereafter as Chief of Service until October 29, 1918, at the peak of the influenza epidemic at Camp Cody, when he was transferred to Base Hospital No. 134, mobilizing at Camp Wadsworth, S. C., for overseas service. He remained there as commanding officer until December 14, 1918. While at Camp Cody he published with Anthony Avata, Lieut., M. C., favorable results of treating facial erysipelas by a method of collodion circumscription (*Jour. A. M. A.*, Sept. 14, 1918). The armistice having been signed, Base Hospital No. 134 was shortly demobilized, and Major Woodyatt served thereafter as Chief of Medical Service, U. S. Army General Hospital No. 28, Fort Sheridan, Illinois, December 18, 1918, until discharged from service May 22, 1919.

Dr. Russell M. Wilder was a member of the Medical Reserve Corps before the war, and was called to service June 13, 1917, with the rank of captain, being sent to Ft. Benjamin Harrison, Indiana, December 31, at first assistant adjutant. He was transferred to the regular army and sent to France, arriving there January 7. He received a lieutenant's commission on January 12. He was located with Evacuation Hospital No. 2, Baccarat, Vosges Mountains, until September 1, 1918, when he was made Chief of Medical Clinic and Organizer of the Gas Hospital in Toul. From there he was sent on September 28 to take command of the temporary gas hospital in the field behind the Argonne lines. On October 27 he went to the headquarters of the Second Army in Toul, as medical gas officer of the Second Army. He served in this capacity until after the armistice was signed. He was in medical service from that time on until he was discharged.

The occurrence of "war edema" having been observed and studied in Roumania by Dr. Wells, and similar conditions being said to occur in the children of the poor of Chicago, especially since the increased cost of milk and other foods had led to a reduction in the nourishment

of poor children, study of this problem was undertaken by Dr. Maria B. Maver, associated with Dr. W. H. O. Hoffman and Dr. Wells. Experiments were devised to determine just what changes in diet will relieve this condition. These experiments were continued despite the cessation of the war because of their general significance and the results will soon be ready for publication.

Dr. W. D. Sansum took charge of Dr. Woodyatt's work during his absence in military service, conducting the diabetic clinic, teaching medical students enlisted in the medical corps, and continuing the research work of the laboratory. August 1, 1918, he was elected to Advisory Board No. 3 A, Presbyterian Hospital, and served until the close of the war. In the summer and fall of 1918, he investigated the toxicity of the various explosives: Tetryl, trinitro-meta-xylol and tetra-nitraniline. The results of this work will eventually be reported together with that of the other laboratories.

Mr. Verne Eastman, Fellow in the Institute, assisted Dr. Sansum in the work with toxic explosives.

Dr. E. J. Witzemann continued his work in the laboratory until June 1, 1918, when he received an urgent call to go to the American University Experiment Station of the Bureau of Mines, in Washington, D. C. (this station became a part of the Research Division of the Chemical Warfare Service, U. S. Army about July 1, 1918) to direct research on an important war gas which was to be manufactured on an enormous scale at once. The research results that had been turned over to the chemical engineers had been proved useless for purposes of manufacture on a large scale, and all had to be done again with the greatest possible speed. Research chemists were scarce. Twenty green chemists were broken in and set to work in a large open shed on account of the very poisonous character of the gas and its raw materials. In two weeks, by working in three shifts and in close conference with the engineer in charge, the main problem was solved. In seven more weeks all important questions relating to manufacture, stability and control of plant product had been solved, and several entirely new and cheap methods of manufacture had been developed in the laboratory. This gas is said to be the one that is frequently spoken of as having been perfected just before the armistice was signed, and since its nature and properties have not been announced by the War Department, it will not be discussed further. After completing the above, Dr. Witzemann directed research in conjunction with the same chemical engineer (Dr. J. R. Withrow, professor of Industrial and Engineering Chemistry, Ohio State University) on methods of manufacture of raw materials for a solid war gas for naval use. When the armistice was signed he was in the midst of the preparation of a comprehensive compiled report of research on the first gas problem previously submitted, and upon completion of this his resignation was approved for December 31, 1918.

On the entrance of the United States in the war the workers connected with the Sprague Laboratory of the Children's Hospital, held themselves at the disposal of the National Research Council, itself a part of the National Council of Defense. At the request of the director of this board, they studied the influence on experimental gas bacillus infection of substances giving off oxygen more or less easily. It was thought that oxygen liberated at the seat of infection might kill the bacilli and at the same time exercise a beneficial effect on the lesions. The results showed that such was not the case. The production of antitoxic sera by Weinberg, Bull and others made a further study along the lines planned inadvisable, the more so as the negative results were in agreement with the results obtained elsewhere with oxidizing substances. This work was done in June, 1917, by Drs. S. Amberg and H. F. Helmholz with the assistance of Miss Carol Beeler and Dr. B. Rappaport.

Late in December, 1917, Dr. Reed Hunt, professor of pharmacology in Harvard University, asked Dr. Amberg to take part in the investigation of certain aspects of the "mustard gas" (dichlorethylsulfide) problem. Dr. Hunt was a member of the advisory board of the gas defense service, a service conducted at that time under the U. S. Bureau of Mines. His duties included the assignment of pharmacological problems to various investigators. The problems assigned concerned the skin lesions produced by dichlorethylsulfide. The work started early in January, 1918. The results were incorporated in a number of official reports transmitted at first to Dr. Hunt and by him to the board. When the gas defense service was taken over by the War Department as a branch of the Chemical Warfare Service the reports were transmitted to Capt. (later Major) H. C. Bradley, of this service.

The number of individual experiments was considerably over one thousand. The work may be outlined briefly as follows:

1. A method was devised by which lesions of like intensity could be produced at will in the rabbit. Obviously, this is a necessary prerequisite when the influence of drugs or other therapeutic measures is to be studied.

2. The method was then applied to man. In this way a basis was laid for judging the efficacy of therapeutic or protective measures in man, from the results obtained in rabbits.

3. The histological changes produced by dichlorethylsulfide on the skin of rabbits were studied at various periods after exposure.

4. A large number of substances were tried for their possible therapeutic effect on the burns. Experiments which seemed to give promise in the case of rabbits were repeated on man with similar results.

5. At the special request of the board a number of ointments were tried with reference to their protective value.

6. Another phase of the problem of protection was the preparation of fabrics impenetrable to the sulfide in liquid or gaseous form and to other substances of similar character. Here very good results were obtained.

7. Several other phases of the problem were attacked, e. g., the question of eye irritation by the blister contents of the burns; the treatment of suitable burns in man by adhesive tape.

Besides the work briefly outlined above, experiments were conducted for the Committee on Industrial Poisoning in Munition Workers with a new explosive which was highly toxic. The effect of fumes given off at various degrees of temperature was studied. Here also the effect on the skin of rabbits and man was the principal subject of investigation as well as protective measures.

For the human tests in all this work only volunteers were used. Tests were made on S. Amberg, R. S. Austin, W. O. Bayard, Carol Beeler, H. F. Helmholz, H. L. Lussky and W. B. McClure.

Connected with the work were: S. Amberg, R. S. Austin, Carol Beeler, H. F. Helmholz, Mary E. Maver, H. O. Lussky, Katherine Mayer, W. B. McClure and L. W. Sauer.

Dr. Helmholz's connection with work extended from the beginning to the end. In November, 1918, he was appointed Consulting Pharmacologist to the War Department.

Dr. Austin, pathologist of the Children's Memorial Hospital, who assisted in this work, left in August, 1918, having accepted a commission in the Medical Corps.

Dr. McClure, having started work on war gases in April, left for the same reason in September. Miss Beeler, who began this work in January, left in June, entering the Medical Department of the U. S. Army, and served as a bacteriologist and technician in the hospital at Camp Sherman.

Dr. Sauer, who started in April, and Dr. Lussky in March, both remained to the end, as did Miss Maver, who began her work in August.

Miss Maver was associated with the work at the Sprague Laboratory of the Children's Memorial Hospital until May, when she left for Madison, where she and Dr. Amberg joined the group of workers engaged in the investigation of problems connected with war gases. Dr. Amberg had gone to Madison in March, 1918. In July the station in Madison, hitherto under the Bureau of Mines, was transferred to the War Department and was known as the "Wisconsin Section of the Medical Division of the Chemical Warfare Service." It was in charge of Major J. A. E. Eyster, M. C., professor of physiology in the Uni-

versity of Wisconsin. Dr. Amberg became identified with this service as pharmacologist in September and Miss Mayer as assistant pharmacologist in November, the appointments terminating with the end of December. At the request of Major Eyster, both continued their war work in Madison until the winding up of the station in April, 1919.

One of the main parts of the work in Madison concerned the determination of the minimal harmful dosage of certain war gases at prolonged exposures, and the recognition of the first signs of ill effect. These experiments were done on dogs. The importance of such studies, particularly with reference to the protection of the working men, need not be pointed out. Such experiments last a week and go day and night. Before the necessary number of privates reported for duty these experiments were at times quite exacting. The study of the physiological pathology and the treatment of phosgene intoxication was another of the problems of the station which required long stretches of continuous work. The protection of the skin against the burning effect of several substances in liquid and gaseous form was the subject of quite extensive experimentation. The general toxic effect of such substances when applied to the skin in liquid or gaseous form was studied, as well as the means of its prevention. The physiological pathology of several substances was determined, and finally a comparative study of the vapors of two gases was conducted. This took into consideration the minimal dosage of each required to produce lesions of the eyes in animals and to produce lesions of the skin in man and animals. Miss Mayer and Dr. Amberg were among the volunteers for the human tests.

Three of the Fellows of the Institute resigned from the staff of the Children's Hospital to enlist in the Medical Corps of the U. S. A. First Lieut. V. D. Greer, who was invalided out of service shortly after he entered; First Lieut. Benjamin Rappaport, who was assigned to duty in Camp Riley and later in Newport News; First Lieut. W. B. McClure, who was assigned to duty at Fort Sheridan.

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THE PHARMACOLOGY AND TOXICOLOGY OF COPPER SALTS OF AMINO ACIDS

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS XVI

HARRY L. HUBER

From the Department of Pathology of the University of Chicago and the Otho S. A. Sprague Memorial Institute

Received for publication March 11, 1918

Since the middle of the nineteenth century certain events have stimulated at irregular intervals the investigative work on copper, until now a remarkably voluminous and contradictory literature has developed. At first the chief interest in copper was its reputed harmfulness to the health of man when used in the greening of certain vegetables for market, and this practice led, after many years of investigation by the French government to the passage of a law prohibiting the use of copper in canned vegetables. Later, however, this law was repealed as the evidence for the harmful influence of copper when taken in such small doses was inconclusive, but in 1911 in the United States, Taylor, Long and Chittenden reported some work to show that "even such small quantities of copper (10 to 12 mgm.) may have a deleterious action and must be considered injurious to health."

In France again the work on copper received another impetus in the early 80's when it was found by Millardet to be an effective agent for combating certain plant growths which attacked vineyards. After this discovery various preparations of copper were used as germicidal agents and after the work of Naegeli on Spirogyra, copper came into common use as an effective agent in combating vineyard diseases and in the purification of city water supplies. The more recent contradictory works of Moore,

and of Clark and Gage show that there is still a question as to the value of copper in the purification of water supplies.

Although copper has been recommended for many years as a therapeutic agent in such diseases as typhoid (Burg, Walker, Wilcke), cholera (Cummins, Burg), cancer (Loeb) (Manara), diarrhea and dysentery (Cummins, Gresswell), leprosy (Takano), actinomycosis, sporotrichosis (Bevan), etc., it is only in recent years that an attempt has been made to study systematically the therapeutics of various copper compounds. In 1911, Corper began in this laboratory a series of experimental studies on the chemotherapy of tuberculosis, using some copper salts of amino-acids, but his work failed to show any specific curative effects with the copper salts employed. A year or two later von Linden, Strauss, Meissen, Bodmer, and Eggers reported very striking results in the treatment of pulmonary and skin tuberculosis by using a specially prepared copper-lecithin compound. Pohl, Benzi, and others, however, could see no improvement in their tuberculous patients after using this or similar preparations. Recently Kogo, Sato, Omura and other Japanese investigators have reported marked improvement in cases of pulmonary and skin tuberculosis after using a preparation of copper and cyanide which they call cyano-cuprol. This work is still too new to be either accepted or rejected.

Experimental work has shown that the value of copper as a fungicidal, bactericidal, or therapeutic agent depends on bringing the copper in contact with the organism to be destroyed in the proper form and concentration. Certain preparations have been found very effective in destroying fungus growths, and Bordeaux mixture is commonly used for this purpose, but Malverzin and Perrin recommended other copper compounds which are said to be more effective, although Bedford and Pickering, after studying the effects of many fungicides, come to the conclusion that the effectiveness depends entirely on the concentration of copper. There is also a discussion as to the bactericidal properties of different copper salts. Gildersleeve found copper sulphate to be more active, due to its more rapid electrolytic dissociation, copper chloride, copper acetate and copper

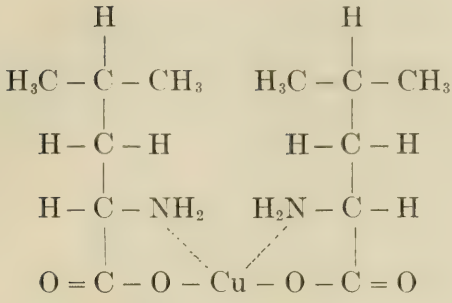
nitrate following in the order given. This finding is in accord with that of Krönig and Paul. Shaw and Mackenzie report that copper-alanine is very lethal in low concentrations to the lower organisms, while DeWitt and Sherman were unable to find much difference in the activity of copper sulphate and copper chloride on certain organisms. A great deal of work has been done on experimental animals to determine the action of different copper compounds but the methods of experimentation are so variable that comparisons are hard to make.

Thus, Taylor found copper-oxide, copper-tartrate and copper-phyllocyanat equally toxic for rabbits, Lehmann found that copper acetate, sulphate, carbonate, citrate, chloride and succinate differed in no way in toxicity. Mock found no difference in the action of copper fatty acids and other copper salts, while Baum and Seeliger found copper oleate most toxic, copper acetate and copper sulphate somewhat less toxic, and copper-haemol almost without action. Brandl found copper oxydnatron most toxic, then copper acetate oleate and stearate in the order given. Tschirch and Lang were unable to see any difference in the different copper compounds. Feltz and Ritter decided that the acetate was more toxic than the sulphate and that the insoluble forms of copper albuminate were without action. Filehne also found that cupratin (made by heating sodium albuminate with copper sulphate) was almost harmless and says, "It seems that foods containing copper combined with albumins are harmless." Filehne also found that copper-sodium-tartrate was more toxic internally and less toxic intravenously than copper-potassium-tartrate. Burq and Ducom found on effect from feeding metallic copper while De Moor found it toxic. Trolldenier noted that copper oleate produced greater pathological changes than other copper salts. Uhl prepared a copper peptone compound which he found to be almost non-toxic. Long and Taylor reported that there was a distinct difference in the action of copper when given as the sulphate and as the compound found in greened vegetables. The different results obtained by these various observers are hard to explain, for it seems that copper, like mercury and other heavy metals, must form compounds which vary in toxicity.

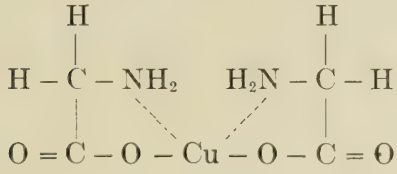
In order to throw more light on some of these questions and to compare the toxic properties of copper sulphate with some of the copper compounds of pure amino-acids, (glycine, glutaminic acid, and leucine), the following experimental work was undertaken. Amino acid combinations with copper were chosen for several reasons: No similar experimental work has thus far been reported, to our knowledge, in which copper compounds of pure amino acids have been used; the amino-acid radicals are utilizable by the organism and their use eliminates the introduction of radicals which may modify the reaction; and the therapeutic value of copper, as indicated by the reports of recent investigators, may be enhanced by its introduction in combination with radicals which are non-toxic and are easily utilizable. It was thought that possibly amino acid salts of copper might be more readily absorbed, or penetrate the cells better, or be less toxic than other salts, since the acid radical is one constantly present in the blood and tissues. To insure uniformity practically all the work was done on guinea pigs. This work naturally falls into two parts, the investigation of the acute toxic properties and of the chronic toxic properties of these compounds.

PREPARATION OF THE COPPER COMPOUNDS

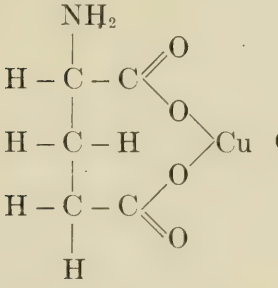
Four copper salts including one inorganic preparation, copper sulphate, and three organic preparations, copper glycinate, copper glutaminate, and copper leucinate, were used in the experimental work. The organic preparations were chosen because they represent an easily soluble and a difficulty soluble double amino-acid copper complexes, copper leucinate and glycinate, and a single amino-acid copper complex, copper glutaminate. The formulae for these compounds are:



Copper leucinate



Copper glycinate



Copper glutaminate.

These organic preparations of copper were prepared in this laboratory. The leucine was prepared by hydrolysis of proteins by Dr. Koch of the physiological chemistry department. The glycine was prepared synthetically by Dr. Witzemann of the Sprague Institute and the glutamic acid prepared by myself from wheat glutin. As the glutamic acid is isolated in the form of glutaminic hydrochloride, it is necessary to remove the chlorine before making the copper salt. The chlorine was removed with an excess of freshly prepared Ag_2O and the excess Ag precipitated with H_2S. From this point the preparation was the same as with the glycine and the leucine. These amino acids were dissolved in a large excess of water and were brought to a boiling temperature. To this was added freshly prepared,

sulphate-free CuO in boiling water. The mixture was boiled and stirred for fifteen to twenty minutes, then filtered to remove excess of CuO. These solutions were then evaporated on the steam bath until crystallization began, then were cooled and the crystals collected by filtration. The mother liquor was evaporated further and other crops of crystals collected. The crystals were then dried and kept in a dessicator until used. The leucinate and the glycinate form blue salts and the glutaminate a greenish salt. According to Kober and Sugiura the mono-basic copper-glycinate is soluble, the mono-basic copper-leucinate very insoluble and the dibasic copper-glutaminate soluble.

METHOD OF MAKING COPPER DETERMINATIONS

The method of making copper determinations was that used by Corper, with slight modifications. The organic material was destroyed completely, as described by Corper, in Kjeldahl flasks with nitric and sulphuric acids, and the sulphuric acid evaporated to a small volume by forcing air down the neck of the flask. This mixture was then washed into a 200 cc. wide-mouthed bottle with copper-free redistilled water, and neutralized to litmus with copper-free 50 per cent sodium hydroxide. After neutralization, one cc. of sulphuric acid (concentrated sulphuric acid diluted with an equal volume of water) and sufficient distilled water were added to bring the volume to about 80 cc. The solution was then heated to about 70°C. and the copper separated from the heated solution by electrolysis. A piece of platinum foil about 2 cm. square suspended by a platinum wire through a small hole in the side of the bottle was used as a depositing electrode. The other electrode was a platinum disc 1.5 cm. in diameter, so arranged that it could be rotated several hundred times a minute. This rotation insured complete mixing of the electrolized solution, and, with a storage battery current of 2 to 2.5 volts and 0.2 amperage, the small amounts of copper were completely deposited in two to three hours, as shown by controls.

After the deposition was complete the method as used by Corper was followed. In all of this work care was taken to

use only copper-free reagents so that quantitative accuracy to about 0.03 mgm. of copper was possible. Recently, Teruuchi and Omura have devised a new method of making copper determinations, which they claim is quantitatively accurate to 0.01 to 0.003 mgm. of copper.

THE EFFECT OF COPPER ON THE EYE OF RABBITS

The minimal irritating concentration of copper sulphate, copper-glutaminate, copper glycinate, and copper leucinate on the eye of rabbits was determined by dropping 1 cc. of distilled water solutions of these salts, made up with different definite concentrations of copper, into the conjunctiva. The amount and rapidity of inflammation and the effect on the lacrimal gland served as an index of the degree of irritability. There was a slight variation in different rabbits and in the same rabbit at different times but, taken as a whole, the results as given in table 1 indicate that the degree of irritability depends on the concentration of copper and not on the salt used, as the same concentration of copper in all the different salts, produced effects which were not distinguishable. It will be noted that the higher concentrations of copper-leucinate and of copper glutaminate are not used, as these salts are not easily soluble in distilled water. All of the higher concentrations produced lacrimation within a few seconds.

TABLE 1

The effect of copper on the eye of rabbits

	CONCENTRATION OF Cu			
	1 per cent	0.1 per cent	0.05 per cent	0.01 per cent
Cu-sulphate	Hyperemia in 2 minutes	Slight hyperemia in 5 minutes	Slight hyperemia 8 minutes	No effect
Cu-glutaminate			Slight hyperemie 8 minutes	No effect
Cu-glycinate	Hyperemia in 2 minutes	Slight hyperemia in 5 minutes	Slight hyperemia 8 minutes	No effect
Cu-leucinate				No effect

THE LOCAL TOXIC ACTION OF COPPER

In 1914 Corper reported the use of the intracutaneous injection method to determine differences in the local toxic action of chemicals. This method was used in studying the local toxic action of copper. These intracutaneous inoculations of 0.2 cc. were made with a 1 cc. tuberculin syringe, in the backs of white guinea pigs, varying concentrations (1., 0.1, 0.01, 0.001 per cent copper concentration) of a water solution of copper sulphate being used on one side of the back and corresponding concentrations of water solutions or suspensions of the copper-amino-acids on the other side of the back, equal concentrations opposite each other. Before inoculation, the hair was shaved from the back and the thickness of the skin at each point of injection was determined by pinching up the skin in a fold and measuring with a micrometer. After the inoculation, daily observations and measurements were made for five to seven days. Some of the animals were killed within forty-eight hours after inoculation and the inoculated areas preserved in Zenker's fluid for histological examination. The higher concentrations of both the copper sulphate and the copper amino-acid always caused a necrosis of the tissue, and varying degrees of induration at the point of inoculation. The lower concentrations caused no necrosis and little or no induration. The gross comparisons did not show any marked differences in the reaction of the equal copper concentrations of the different copper salts. In a few cases the copper-amino acid solution produced a more hemorrhagic lesion, but this was not constant. The degree and kind of necrosis seemed to be alike with the higher concentrations of all the preparations used. No differences in reaction could be made out histologically, thirty-six hours after the inoculation. With all concentrations of all the preparations there was a slight edema of the lower layers of the corneum and a more or less marked polymorphonuclear infiltration and edema in the corium adjacent to the inoculated area. With the higher concentrations of all the preparations there was evidence of beginning degeneration in the corneum at the site of inoculation and the capillaries in

the corium surrounding the area were more distended than with the lower concentrations. Preparations of inoculated areas, taken seven to eight days after inoculation, showed no changes with the lower concentrations and only scar tissue with the higher concentrations.

So far as the local toxic action of the different copper preparations on the skin is concerned, it seems that there are no distinguishable differences in reaction.

SUBCUTANEOUS INJECTIONS OF COPPER

Filehne, Brandl, Pinkus, Sellei, De Moor, Uhl, Chittenden, Corper and others have determined the toxic dose of many copper preparations but, as most of them fail to state definitely all the conditions of their experiments, it is impossible to interpret their findings. Table 2 shows briefly the results of their experiments.

As these findings seem to show that different copper preparations have different degrees of toxicity, a series of experiments was conducted to determine the toxic dose of copper sulphate, copper leucinate, copper glycinate, and copper glutaminate for guinea pigs. These copper solutions, made up with distilled water and calculated in percentage of copper, were injected into the subcutaneous tissue of the axilla and groin. As the higher concentrations of copper solutions cause a necrosis at the point of injection and as Novy has recently shown that relatively large amounts of pure distilled water may be injected even intravenously without harm, an attempt was made to insure complete absorption of the copper by making higher dilutions than have been used by most of the other workers. This made it necessary to make injections at several points and consequently insured a more rapid absorption. The symptoms following the injections when death occurred within ten to twelve hours were the same for all of the copper preparations used; namely, marked irritation and restlessness for about an hour; then a huddling together, the hair on the neck and back standing erect, until death intervened. A few of the pigs developed marked spasmodic contractions of the hind legs and a few which were

TABLE 2

Determinations of the toxic dose of copper

OBSERVER	DATE	COPPER PREPARATION	ANIMAL	METHOD	MILLIGRAMS OF Cu PER KILO	LIVED
		Copper K tartrate			65 0	
		Copper Na tartrate			30 0	
Filehne	1895	Copper Na tartrate	Rabbit	Intravenous	3 0	1½ hours
		Copper Na tartrate	Rabbit	Subcutaneous	9 0	24 hours
		Copper Na tartrate	Rabbit	Per os	50 0	
Brandl	1896	Copper Na tartrate	Dog	Intravenous	3 0	
		Copper Na tartrate	Dog	Subcutaneous	21 0	4 days
Sellei	1913	$CuCl_2$	Guinea-pig	Subcutaneous	50 0	5 to 8 hours
		$CuSO_4$	Guinea-pig	Subcutaneous	25 0	36 to 48 hours
Chittenden	1913	$CuSO_4$	Rabbit	Intravenous	7 5	35 minutes
		$CuSO_4$	Rabbit	Intravenous	4 1	4 hours
		$CuSO_4$	Dog	Intravenous	1 4	
Corper	1914	Copper amino-acid mixture	Mice	Intraperitoneal Intramuscular	5 0-10 0	
Pinkus	1914	$CuSO_4$	Guinea-pig	Intravenous	1 5	
		$CuSO_4$	Guinea-pig	Subcutaneous	17 0-20 0	
		$CuSO_4$	Guinea-pig	Per os	80	
Uhl	1914	Cu peptone	Rabbit	Subcutaneous	23	No effect
		Cu peptone	Rabbit	Intravenous	20	No effect

given larger doses of copper developed paralyses of both hind legs. This latter observation has been noted by several of the early workers (Filehne, Tschirch), and led them to believe that copper is a neuro-toxin, but as these paralyses develop only occasionally, this conclusion does not seem justified. The autopsy findings in pigs dying within ten hours after the injection show no constant variations for the different copper salts. A few typical protocols will give the usual findings:

Guinea-pig. Weight 500 grams. Injected 8 mgm. Cu as copper glutaminate per kilo in 18 cc. of distilled water. Died three hours after injection. No spasmodic contractions—no paralyses. Autopsy findings: Slight edema at points of injection, no excess fluid in peritoneal or pleural cavity, gastro-intestinal tract hyperemic, petechial hemorrhages in serosa of small intestine, gall bladder distended, urinary bladder empty, kidney, liver, spleen, lungs, and heart show no changes.

Guinea-pig. Weight 520 grams. Injected 8 mgm. Cu as copper glycinate per kilo in 17.5 cc. of distilled water. Died sixteen hours after injection. No spasmodic contractions, no paralyses. Autopsy findings: Marked edema of abdominal wall at site of injections, peritoneal exudate 15 cc. hemorrhagic, gastro-intestinal tract hyperemic, no petechial hemorrhages. Gall bladder empty, urinary bladder 2 cc. of a blood tinged urine, kidneys pale, no changes in other organs.

Guinea-pig. Weight 860 grams. Injected 8 mgm. Cu as copper sulphate per kilo in 28 cc. of distilled water. No spasmodic contractions, no paralyses. Died three and one-half hours after injection. Autopsy findings: Slight edema at points of injection, peritoneal exudate (15 cc.) clear, gastro-intestinal tract hyperemic, gall bladder empty, urinary bladder empty, no petechial hemorrhages, no changes in kidney, liver, spleen, lungs or heart.

Guinea-pig. Weight 430 grams. Injected 8 mgm. Cu as copper leucinate per kilo in 17 cc. of distilled water. No spasmodic contractions, no paralyses. Died three hours after injection. Autopsy findings: Slight edema at points of injection, no peritoneal exudate, hyperemia of gastro-intestinal tract, no petechial hemorrhages, bladder distended, urinary bladder empty, no changes in kidney, liver, spleen, lungs or heart.

The only constant difference in findings with the various salts was the greater edema at the injection point produced by the

copper glycinate. Table 3 shows the effect of varying the dosage, and the dilution in these subcutaneous injections.

Some observers (Brandl, Lehmann, Philippeaux) have noted that smaller doses of copper are more quickly fatal in fasted animals than in well fed animals. In the few animals tried here the reverse was true with subcutaneous injections, the well fed animals, with one exception, dying in half the time required with the fasted animals.

In order to show the effect of dilution on the toxic dose of copper, two pigs were injected with equal volumes of a copper glutaminate solution containing 0.011 per cent of copper, and one was injected with a solution containing 0.033 per cent cop-

TABLE 3

Acute intoxication with copper

DILUTION	Cu	Cu-GLYCINATE	Cu-GLUTAMINATE	Cu-LEUCINATE	Cu-SULPHATE
per cent Cu	*mgm. per kilo*				
0.020	10	Died in 1 hour	Died in 8 hours	Died in 4½ hours	Died in 1 hour
0.020	8	Died in 16 hours	Died in 3 hours	Died in 3 hours	Died in 3 hours
0.020	4	Died in 42 hours	Died in 41 hours	Died in 18 hours	Died in 34 hours
0.020	4	Died in 42 hours	Died in 80 hours		
0.020	4		Died in 90 hours		
0.032	6	Died in 35 hours	Died in 50 hours		
0.032	6		Died in 72 hours		
0.032	6		Died in 26 hours		
0.032	6		Died in 20 hours		

per. The pigs receiving the higher dilution died on the third day, while the pig receiving the lower dilution apparently recovered from the effect of the injection and was killed on the fifth day on account of the large area of necrosis at the places of injection. The autopsy findings in these pigs differed greatly, there being no changes in the viscera of the pig receiving the lower dilution, while in those receiving the higher dilutions the hyperemic intestinal tract and parenchymatous changes in the kidney, spleen and liver, were very evident.

Klemptner and others have found appreciable amounts of copper in the blood following injections of copper salts. They

have reported that no trace of copper could be found in the serum but all in the blood cells. Chemical examinations of the blood of eight pigs which had been injected subcutaneously gave results as shown in table 4. The blood was drawn from the heart six hours after the injection.

In two of the pigs, one injected with copper sulphate, the other with copper glutaminate, there was an hemorrhagic peritoneal exudate which on analysis showed a copper content of 0.01 mgm. for the former and 0.059 mgm. for the latter. The autopsy findings in all these pigs are the same as given above.

Chemical analyses for copper were made on the viscera of some pigs which were injected subcutaneously with the lethal dose of the four copper salts. The urine and feces were collected for a period of four days before injection, and the urine

TABLE 4

Blood analyses for copper after subcutaneous injection

	COPPER SALT							
	$CuSO_4$		Cu glucinate		Cu glutaminate		Cu leucinate	
Milligram of Cu injected per kilo	4.0	8.0	4.0	8.0	4.0	8.0	4.0	8.0
Blood analyzed (cubic centimeter)	6.0	16.0	10.0	10.0	8.0	5.0	8.0	5.0
Copper found	0.0	trace	0.0	0.0	0.0	0.0	0.0	0.0

after the injection, and copper analyses made on these collections. The results of these analyses are given in table 5.

The autopsy findings in all these pigs are similar to those given above. There were no feces in any case after the injection and in pigs 3, 6, 7, 9 and 10, the urine after the injection appeared bloody. As so much copper was found in the feces and in the gastro-intestinal tract, analyses of specimens of the oats and carrots which were fed to the pigs were made and appreciable amounts of copper were found in both, 4.2 mgm. of copper per kilo in oats and 1.92 mgm. per kilo in carrots. These figures are somewhat lower than those reported by other investigators, but many have shown that regional differences exist.

Taken as a whole, this series of experiments indicates that

TABLE 5

Copper in the viscera after subcutaneous injections

	COPPER SALT									
	Control		Cu-leucinate		Cu-glutaminate		Cu-glycinate		Cu-sulphate	
Pig number	1	2	3	4	5	6	7	8	9	10
Milligram of Cu injected per kilo			12	10	12	10	12	10	12	10
Death after injection			4 hours	4½ hours	7 hours	8 hours	5 hours	1 hour	7 hours	14 hours
Total milligram of copper in feces before			0.326	0.356	0.174	0.122	0.732	0.136	0.538	0.033
Total milligram of copper in urine before			0.006	0.000	0.000	0.000	0.001	0.000	0.022	0.00
Total milligram of copper in urine after			0.035	0.006	0.000	0.000	0.396	0.000	0.302	0.198
Total milligram of copper in stomach and contents			0.196	0.137	0.148	0.162	0.191	0.174	0.149	0.078
Total milligram of copper in large intestines and contents			0.296	0.382	0.332	0.221	0.198	0.410	0.628	0.356
Total milligram of copper in small intestines and contents			0.280	0.252	0.245	0.292		0.502		0.220
Milligram of copper per kilo in:										
Lung	0.000	0.000	0.020	0.05	0.001	0.02	0.003	0.001	0.06	0.000
Spleen	0.000	0.000	0.000	0.000	0.000	0.000	0.01	0.000	0.49	0.000
Heart	0.000	0.000	0.001	0.032	0.000	0.000	0.018	0.000	0.07	0.000
Kidney	0.010	0.000	0.020	0.510	0.240	0.640	0.410	0.210	0.49	0.420
Liver	0.045	0.035	0.136	0.160	0.135	0.180		0.180	0.17	0.380

there are no differences in the action of the copper salts used, although the leucinate in a few cases seemed more toxic. The lower copper value in the feces of pigs 6, 8, and 10, is probably due to a change in the diet to new carrots, during the experiment. The finding of copper in the urine of pigs 3, 4, 7, 9, and 10, after the injection shows that the kidneys excrete small quantities of this metal. This observation has been made by others, while Chittenden never found copper in the urine of experimental animals that were fed copper preparations although the kidneys showed an appreciable amount of copper. The urine of pigs 3, 7, and 9 showed traces of copper before the injection and much larger amounts after the injection. This may mean that the pigs were utilizing a part of the copper in the normal diet. Pigs 4 and 8 did not excrete urine after the injection. The finding of large amounts of copper in the alimentary tract and its contents is of uncertain value, as the foods which were used contained some copper. The analyses of the other viscera, however, although not showing constant variations for the different salts used, show that the liver and the kidney are the chief organs in which copper is found; in all cases but one the kidney containing a higher percentage of copper than the liver. The blood analyses show that the injected copper disappears very rapidly from the blood stream. Klemptner has found in experiments *in vitro* that the copper is attached to the red blood cells and is not in the serum. If this is true, the red blood cells to which the copper is attached must be removed from the blood stream within a short time.

DIALYSIS OF COPPER SALTS THROUGH CELLOIDIN SACS

Kober and Sugiura have noted that the copper salts of the amino acids are dialyzable and Filehne has reported that there are marked differences in the rate of dialysis of certain copper compounds. Many observers have reported that copper easily forms complex protein molecules when brought in contact with proteins, and experimentation has shown that when vegetables are greened with copper two complex copper compounds are

formed. The question naturally arises as to the fate of copper introduced directly into the blood stream or taken up after injection or from the gastro-intestinal tract. Does the copper form a complex protein compound and, if so, is this compound able to penetrate cell membranes? In order to throw more light on the action of certain copper compounds a series of

TABLE 6

Dialysis of copper salts with serum

	COPPER SALT							
	Cu-leucinate		Cu-glutaminate		Cu-glycinate		Cu-sulphate	
Milligram of Cu used.....	0.402	0.403	0.472	0.476	0.468	0.460	0.516	0.506
Horse serum inside (cubic centimeters)	10	10	10	10	10	10	10	10
Horse serum outside (cubic centimeters).....	33	33	33	33	33	33	33	33
Per cent of Cu dialyzed...	59.8	58	60	58.7	57.8	5.9	58.4	59.7

TABLE 7

Dialysis of copper salts with distilled water

	COPPER SALT							
	Cu-leucinate		Cu-glutaminate		Cu-glycinate		Cu-sulphate	
Milligram of Cu used.....	0.402		0.472		0.464		0.508	
H_2O inside (cubic centimeters	10	10	10	10	10	10	10	10
H_2O outside (cubic centimeters	33	33	33	33	33	33	33	33
Per cent of Cu dialyzed...	71.5	70.4	67	68	70.8	71	68	70

dialysis experiments was conducted. The copper salts were made up in the same molar concentration and dialyzed in serum and in distilled water solutions. In one series horse serum was used on both sides of the celloidin sac and in another series distilled water was used on both sides. In both series the copper was added to the serum and to the distilled water and incubated at body temperature for six hours, then was transferred to the dialyzing sac and incubated for twelve hours at body temperature. Analyses were then made to determine the amount of copper in the fluid outside the dialyzing sac. The results of the dialyses are given in tables 6 and 7.

Slight variations with the same salt are probably due to slight differences in the thickness of the dialyzing sacs. The figures show that there is no appreciable difference in the rate of dialysis with the four salts tested and very little difference between the distilled water and horse serum dialyses. These findings then, oppose the view that these copper salts, when brought in contact with protein materials, form complex protein molecules which are not readily dialyzable. This fact may explain the rapid disappearance of copper from the blood stream as noted above and is also in accord with the finding of copper in peritoneal and pleural exudates after injections of copper.

EXPERIMENTAL CHRONIC COPPER INTOXICATION

The production of a typical chronic copper intoxication has been disputed for many years and even today there are many who state that a chronic copper intoxication cannot be produced. There seem to be good grounds for contention, as those who oppose the view that copper may produce a chronic intoxication point to the numerous examples of workers in copper who show no symptoms of intoxication, even after they are so saturated with copper that their hair, nails, gums, and bones are colored greenish. They contend that the apparent chronic copper intoxication is caused by some other agent and is not due to the copper. On the other hand, numerous experimenters with animals have shown that, in animals, at least, certain definite pathological changes follow long continued ingestion of copper and that these changes are accompanied by the deposition of copper in certain tissues. Taylor has recently shown that small amounts of copper may be ingested for a long period by man without producing any distinguishable nutritional disturbances. However, with reference to the retention of copper in the liver, he says, "I do not believe such a retention of heavy metal can be a negligible matter even in the complete absence of present symptoms referable thereto." As such different conclusions have been drawn by different observers, it is almost impossible to interpret the final results. This discrepancy in findings may

be partially explained by the different methods used by these investigators. Many results can not be interpreted as the observers have failed to state all of the conditions of the experiment. It is necessary to know the dosage per kilo body-weight, the method of ingestion, the species of animal, and the duration of the experiment before any conclusions can be accepted. Table 8 summarizes briefly some of the work bearing on the question of chronic copper intoxication.

This table shows the marked variations in the conditions of the experiments which may explain the different results obtained. Small dosages are recommended by Baum and Seeliger, Lewin, Meyerhardt, Chittenden and others, as it was found that larger amounts of copper were absorbed with small doses than with larger doses and it was assumed that the larger doses produced a chronic catarrhal inflammation of the gastro-intestinal tract which interfered with absorption. The question of the variation in toxicity of the different copper compounds accounts for the difference in dosage used by different workers. Lehmann, Mock, Tschirch, Demme and Lang, and Taylor could find no difference in the toxicity of the different copper compounds that they used, while Feltz and Ritter, Filehne, Brandl and Baum, and Seeliger found marked variation in the toxicity of different salts, depending on the copper preparation used. Because of the different views as to the toxicity of the different copper compounds, it seemed advisable to test the toxicity of the four salts used in this present experimental work by the chronic intoxication method. The experimental part of the work is divided into three parts, the attempt to produce chronic intoxication by (1) subcutaneous injections, (2) intramuscular injections and (3) feeding.

SUBCUTANEOUS INJECTIONS

As I have found no records of attempts to produce a chronic copper intoxication by making subcutaneous injections, the dosage used was calculated on the relative values of subcutaneous injection and feeding doses in the production of acute copper intoxication. Filehne found that this ratio was about

TABLE 8

Chronic copper intoxication by feeding

OBSERVER	DATE	Cu PREPARATION	MILLIGRAMS OF Cu PER KILO PER DAY	LENGTH OF EXPERIMENT	ANIMAL USED	RESULTS
				days		
Ellenberger and Hofmeister	1883	$CuSO_4$	10.0	140	Sheep	Pathological changes in liver and kidney
Ellenberger	1898	$CuSO_4$			Dogs	Pathological changes in liver and kidney
		Cu haemol			Sheep	
		Cu acetate			Cats	
		Cu oleate			Goats	
Filehne	1895	CuNa tartrate	11.0	16	Rabbit	Pathological changes in liver and kidney
	1896	Cu stearate	4.0	60	Dogs	
		Cu metallic	4.0	60	Dogs	
Brandl	1893	CuNa tartrate		15	Rabbit	Pathological changes in liver and kidney
		CuO		37	Rabbit	
		Cu haemol	6.9		Dog	
		Cu oleate	7.7	14	Dog	
		$CuSO_4$		130	Rabbit	
Baum and Seeliger	1898	Cu haemol	7.7–12.5	220	Dogs	Pathological changes in small intestine, liver and kidney
		$CuSO_4$	2.0– 3.5	220	Dogs	
		Cu acetate	0.8– 5.0	220	Dogs	
		Cu oleate	2.5– 3.0	220	Dogs	
Trolldeinier	1897	Cu haemol	3.5	220	Dog	Pathological changes in liver and kidney
		Cu acetate	12.0	28	Cat	
		Cu SO_4	3.0	220	Dog	
		Cu oleate		13	Cat	
Chittenden	1913	Coppered peas	0.30– 0.4	60	Dogs	Pathological changes not noticeable
		Cu SO_4	0.35	60	Dogs	

one to fifteen. Others have found the ratio to be about one to twelve. If the average dose per os to produce a chronic intoxication is taken at 4.5 mgm. of copper per kilo per day, the subcutaneous injection will be about 0.30 mgm. copper per kilo per day. This dose, however, is as large as that given per os by Chittenden but he had a special reason for giving such a small dose. In this series of experiments, injections were made into the loose tissue of the axillae and the groins, being so arranged that every fifth injection was made in the same area. At first the injections were made at four day intervals and later at three day intervals, so that about twenty days elapsed between injections at any one place. The injections were made with a 1 cc. tuberculin syringe, as olive oil emulsions

TABLE 9

Subcutaneous injections of copper

	Cu salt							
	Copper Leucinate		Copper glutaminate		Copper sulphate		Controls	
Pig number	11	12	13	14	15	16	17	8
First weight (grams)	390	400	390	410	280	500	400	310
Weight at death (grams)	620	670	620	700	625	715	640	500
Milligrams of Cu injected	16.7	16.7	16.7	16.7	16.7	16.7	0	0

of the copper salts containing 2.0 mgm. of copper per cc., from May 18 to August 30, the pigs having received in that time about 17 mgm. of copper.

All the pigs at the time of death, one hundred and two days after the beginning of the experiment, were sleek and fat and at no time had there been any ulceration at the points of injection. Three days after the last injection the pigs were killed and tissues from the liver, spleen, kidney, heart and lung preserved in Zenker's fluid for histological examination and the remainder of these organs taken for chemical analysis. The results of the chemical analyses are given in table 10. The autopsy findings showed no gross pathological changes, except that in three pigs the kidneys showed a few small cysts.

TABLE 10

Copper content of tissues after subcutaneous injections

	COPPER SALT							
	Leucinate		Glutaminate		Sulphate		Control	
Pig number	I	II	I	II	I	I	I	II
Milligram of Cu. per kilo in								
Spleen	0.08	0.03	0.05	0.000	0.000	0.000	0.000	0.006
Heart	0.06	0.05	0.13	0.16	0.20	0.12	0.000	0.000
Lung	0.03	0.04	0.04	0.01	0.000	0.008	0.000	0.000
Kidney	0.23	0.24	0.22	0.19	0.09	0.19	0.04	0.000
Liver	1.35	0.69	0.58	0.49	0.55	0.31	0.039	0.036

INTRAMUSCULAR INJECTIONS

The same scheme of injection was used in these experiments as was used in the subcutaneous injections, the only difference being that the inoculations were made into the muscles of the fore and hind legs. The duration of the experiment and the total amount of copper injected were the same as in the subcutaneous experiment and similar examinations and analyses were made. The autopsy findings showed nothing abnormal. The results of the chemical analyses are given in table 11.

TABLE 11

Copper content of tissues after intramuscular injection

	COPPER SALT							
	Leucinate		Glutaminate		Sulphate		Control	
Pig number	I	II	I	II	I	II	I	II
Milligram of Cu per kilo in:								
Spleen	0.15	0.14	0.06	0.13	0.000	0.14	0.000	0.006
Heart	0.03	0.000	0.000	0.000	0.000	0.07	0.000	0.000
Lung	0.01	0.07	0.10	0.10	0.000	0.04	0.000	0.000
Kidney	0.12	0.15	0.06	0.21	0.09	0.11	0.04	0.000
Liver	0.49	0.55	0.69	0.57	0.54	0.87	0.03	0.036

The histological examination of the tissues, liver, kidney, spleen, myocardium and lung, did not show any constant changes when compared with the tissues of control animals. Trolldenier, Ellenberger and Hofmeister, Brandl, Klemptner and De Moor found certain changes, especially in the liver and kidney but

the changes noted by them varied considerably. Some, Smith, Trolldenier, even noticed that the changes were more marked in some salts than in others but in our tissues no differences could be made out either for the different salts or for the different modes of injection. Certain inconstant changes were noted; the Malpighian bodies of the spleen were very prominent in some sections, the lungs showed frequently markedly thickened alveolar walls and many small collections of round cells, the kidneys often contained small foci of round cells and a slight increase in connective tissue in the cortex, but the hemorrhages and brownish granules spoken of by Klemptner, Ellenberger and Hofmeister were absent, the livers seemed less fatty in the fed than in the control animals and the collections of brownish pigments and evidences of periportal inflammation spoken of by many were not observed. Taken as a whole, the findings in these tissues in general, corroborate the findings of Smith, i.e., that the tissue changes are not characteristic, although analyses show that the copper is deposited in the tissues.

These analyses show, as Corper has previously noted, that copper, which is introduced subcutaneously or intramuscularly, is deposited chiefly in the liver and to a much less degree in the kidney.

This deposition in the different tissues is not influenced by difference in the form of copper or by the different methods of introduction and, as shown in the histological report, causes no appreciable changes in the tissue.

FEEDING

The summary of chronic copper intoxication given above shows the marked variation in the dosage of copper per day per kilo used by the different workers where the main purpose of the experiment was to produce a chronic copper intoxication. As the main purpose of the present experiment is to determine whether the different copper salts used show different degrees of toxicity, an average sized dose, based on the work of others, was used, the dose being the same for all the salts. These salts

were fed to guinea pigs in pill form, each pill containing two mgm. of copper with a vehicle of graham crackers, olive oil, flour and water. These pills were fed at first daily, in the morning; then twice daily, morning and afternoon; then three times daily, morning, noon and afternoon. This method of feeding insured at least one feeding per day on an empty stomach, at which time absorption is more complete. Controls were given the same ration, minus the copper. These pigs were fed about 360 mgm. of copper in seventy-two days. At the close of the experimental period the animals were killed by bleeding, examined for gross pathological changes, tissues preserved in Zenker's fluid for histological examination, the other tissues kept for chemical analysis. The following table shows the results of these examinations.

The results of the attempts to produce a chronic intoxication by feeding the copper salts named above, are similar to those obtained by the injection methods, the distribution and amount of copper being practically the same regardless of the method used. The histological picture of the tissues where copper is deposited also fails to show any changes which can be attributed to the copper. Fatty changes in the liver and kidney were very inconstant and showed no relation to the amount of copper deposited. Six of the twelve pigs died from various causes (intestinal intoxication, puerperal infection, advanced tuberculosis) or were killed before the completion of the feeding period and complete examinations of these were made at the time of death. Three of these and three of those killed at the end of the feeding-period had experimental tuberculous lesion in the lungs and two had tuberculous lesion in the liver and spleen. Analyses of these tuberculous organs failed to show a higher concentration of copper than was found in the non-tuberculous pigs. One large caseous nodule in the liver contained only about one-fifth as much copper as the surrounding liver tissue. These findings are not in accord with those of the German investigators who used certain copper combinations in the treatment of skin and pulmonary tuberculosis, but corroborate the findings of Corper.

TABLE 12

Copper content of tissues after feeding

	COPPER SALT													
	Leucinate			Glutaminate			Glycinate			Copper sulphate			Controls	
Pig number	I	II	III	I	II	III	I	II	III	I	II	III	I	II
Time fed, days	18	72	65	44	72	72	72	58	72	51	15	72	72	72
Milligram of copper per kilo in:														
Spleen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.000	0.000	0.00
Heart	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.002	0.000	0.00
Lung	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.005	0.000	0.00	0.00	0.000	0.000	0.00
Kidney	0.00	0.00	0.00	0.03	0.14	0.04	0.15	0.010	0.015	0.01	0.00	0.040	0.001	0.00
Liver	0.59	0.31	0.87	0.56	0.34		0.40	0.700	0.500	0.36	0.26	0.680	0.040	0.03
Blood		0.00			0.02	0.00	0.00		0.010			0.000	0.000	0.00
Changes in weight, in grams	Gain 50	Lost 25	Lost 240	Lost 10	Gain 25	Gain 160	Gain 105	Lost 60	Lost 20	Lost 150	Gain 15	Lost 60	Lost 150	0.00

It is of interest to note that the livers of pigs newly born of mothers on the copper diet contained almost the same proportion of copper that the mothers' liver contained, while the remainder of the body contained only a trace of copper. This shows definitely, as has been pointed out by others, that copper passes through the placenta.

GENERAL SUMMARY

1. Copper, whether in the form of the sulphate, leucinate, glycinate or glutaminate, shows no variations in action when introduced into the conjunctiva of rabbits. The lower dilutions, 1 per cent, produce hyperemia and lacrimation to the same degree, while the higher dilutions are inactive.

2. Copper, in the salt forms named above, shows very little if any variation in ability to produce acute intoxication when introduced in dilute solutions, subcutaneously. The dilution is important as the local injury produced by the higher concentrations interferes with absorption. The toxic subcutaneous dose of all the salts was found to be between 4 to 6 mgm. per kilo for guinea pigs.

3. Copper, in the salt forms named above, shows no variations in action when introduced intracutaneously. The lower dilutions cause necrosis and induration and the higher dilutions cause little or no change. The histological picture with the same dilution of all salts is the same.

4. Copper, in the salt forms named above, shows no variation in action when introduced in small gradually increasing doses, 0.5 to 1 mgm. copper per kilo, for a long period of time, one hundred and five days, either subcutaneously or intramuscularly. This dosage of copper seems to produce no gross or microscopic changes in the liver, kidney, spleen, heart, or lungs, although the liver and kidney both show marked increase in amounts of copper when analyzed.

5. Copper, in the salt forms named above, shows no variation in ability to dialyze through celloidin sacs when either water or horse-serum are used both inside and outside the sac.

The rate of dialysis is somewhat faster with distilled water than with horse serum.

6. Copper, in the salt forms named above, shows no variation in action when introduced by feeding in small gradually increasing doses, to 10 mgm. per kilo per day, for a long time, seventy-two days. The deposition of copper in the liver and kidney and the lack of gross and microscopic changes in the tissues examined, simulate the findings in those experiments where the copper was introduced subcutaneously or intramuscularly.

7. This experimental work seems to show that the three copper amino acids examined produce exactly the same physiological effects as a simple inorganic salt, copper sulphate.

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DISTRIBUTION OF GOLD IN ANIMAL TISSUES

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. XVII

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In a recent experiment on the chemotherapy of tuberculosis, gold, in the form of various salts, was administered to a considerable number of guinea-pigs and the organs and tissues of the animals were subsequently analyzed quantitatively to determine the distribution of that metal. But few complete analyses showing the distribution of different metals in animal tissues have been found in the literature and a comparison of these with the findings in my gold-treated animals may be of interest from the point of view of the biological chemist.

Copper. Huber, in an article which will soon be published in the Journal of Pharmacology and which I have seen only in manuscript, has determined in this laboratory the distribution of copper in the viscera both of animals acutely poisoned and dying within a few hours after administration of the metal and in similar animals which have been subjected to a chronic intoxication. In the acute poisoning, lethal doses of four copper salts were administered, the animals dying in from one to fourteen hours after the injection. The animals subjected to chronic poisoning received nonlethal doses of the same salts at intervals for a considerable time. Table 1 (A) gives briefly the results obtained by him in one set of experiments in which the poisoning was acute, while table 1 (B) gives the results in chronically poisoned animals.

These two tables are given in full for comparison with my analyses. Huber's method of analysis was electrolytic, after the

TABLE 1A

Acute. By H. L. Huber

	COPPER SALT									
	Control		Leucinate		Glutaminate		Glycinate		Sulphate	
	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Milligram Cu injected per kilo			12	10	12	10	12	10	12	10
Death after injection			4 hrs.	4.5 hrs.	7 hrs.	8 hrs.	5 hrs.	1 hr.	7 hrs.	14 hrs.
Total milligrams Cu in feces before injection			0.326	0.356	0.174	0.122	0.732	0.136	0.538	0.033
Total milligrams Cu in urine before injection			0.006	0.0	0.0	0.0	0.001	0.0	0.022	0.0
Total milligrams Cu in urine after injection			0.035	0.006	0.0	0.0	0.396	0.0	0.302	0.198
Total milligrams Cu in stomach and contents			0.196	0.137	0.148	0.162	0.191	0.174	0.149	0.078
Total milligrams Cu in large intestines and contents			0.296	0.382	0.332	0.221	0.198	0.410	0.628	0.356
Total milligrams Cu in small intestines and contents			0.280	0.252	0.245	0.292		0.502		0.220
Milligrams Cu per kilo of liver	0.045	0.035	0.136	0.16	0.135	0.18		0.18	0.17	0.38
Milligrams Cu per kilo of spleen	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.0	0.49	0.0
Milligrams Cu per kilo of lungs	0.0	0.0	0.02	0.05	0.001	0.02	0.003	0.001	0.06	0.0
Milligrams Cu per kilo of kidneys	0.01	0.0	0.02	0.51	0.24	0.64	0.41	0.21	0.49	0.42
Milligrams Cu per kilo of heart	0.0	0.0	0.001	0.032	0.0	0.0	0.018	0.0	0.07	0.0

TABLE 1B

Chronic. By H. L. Huber

	COPPER SALT									
	Control		Leucinate		Glutaminate		Glycinate		Sulphate	
	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Milligrams Cu found in liver	0.039	0.036	1.35	0.69	0.58	0.49			0.55	0.31
Milligrams Cu found in spleen	0.0	0.006	0.08	0.03	0.05	0.0			0.0	0.0
Milligrams Cu found in lungs	0.0	0.0	0.03	0.04	0.04	0.01			0.0	0.0008
Milligrams Cu found in kidneys	0.04	0.0	0.23	0.24	0.22	0.19			0.09	0.19
Milligrams Cu found in heart	0.0	0.0	0.06	0.05	0.13	0.16			0.20	0.12

destruction of all organic matter by means of sulphuric and nitric acids. The copper was deposited on platinum electrodes, then dissolved off and determined by a colorimetric method. Since copper was found in the organs of control animals, the food of the animals was analyzed and found to contain copper in considerable amounts. Corper, by a similar method, made analyses for copper at the end of long periods after the treatment of the animals with copper had ceased, his findings being shown in table 2. From these tables it may be seen that copper is excreted in both the urine and feces, but to a greater extent in the feces. In the acutely poisoned animals, the largest amounts are found in the kidney, the liver usually standing second. In the chronically poisoned animals and in those which received copper by feeding and in those examined after long

TABLE 2

	LIVER	SPLEEN	LUNGS	KIDNEYS	L. GLANDS
47 days after $CuSO_4$	0.007	0.0			0.0
66 days after Cu acetate	0.368	0.0	0.0	0.063	0.0
90 days after Cu oleate	0.4	Trace			
85 days after Cu sulphate	0.0				
69 days after Cu acetate	0.019	0.0	0.0	0.0	
92 days after Cu oleate	0.0				

periods of time, on the other hand, the liver contains far more than any other organ. In fact, in all the long time experiments practically all the copper found was in the liver. Van Itallie and Van Eck have shown by a series of analyses of the livers of patients who had received none of the metal intentionally, that both copper and zinc are found regularly in the human liver, being present in the fetal liver in even larger amounts than at later periods of life. Table 3 reproduces a part of a chart published by them showing the number of milligrams of copper and zinc found in the livers of these patients, whose age and sex are also given. Arsenic was included in this series of analyses, but in only one case was more than a trace found and that individual had been treated with Blaud's pills for some time before death.

Zinc. Blyth reports an experiment in which a dog was poisoned with zinc acetate. He found zinc in the muscles and bones, in the stomach and intestines, in the skin, as well as in the liver, lungs, kidneys and heart. His figures are not given in such a form that I can readily compare them with mine. Most of the zinc seems to have been found in the muscles but there was more in the liver than in any other organ.

Arsenic. Arkins and Corper report the distribution of arsenic in the bodies of animals which had received several doses of various preparations of arsenic, and which were killed within a

TABLE 3

AGE	SEX	ARSENIC	COPPER	ZINC
Still born			26.1	73.9
Some hours			30.0	52.2
5 weeks	m.		8.0	55.7
3 months	m.		18.9	55.0
3½ years	m.	Trace	10.6	67.8
5 years	m.	0.06	2.9	
21 years	f.	0.0	5.7	36.1
24 years	f.	0.0	11.2	79.6
28 years	m.	0.0	4.8	
28 years	f.	0.0	14.8	56.0
32 years	m.	0.03	6.0	50.6
35 years	f.	0.0	5.0	17.7
36 years	f.	Trace	17.7	60.0
37 years	m.	2.63	3.8	54.0

few hours after the last administration of the drug. Table 4 gives briefly the number of milligrams of arsenic per gram weight of tissue found in their animals. Underhill also estimated by the method of Johnson and Chittenden the amount of arsenic in the organs of a woman of twenty years who had died of chronic arsenic poisoning. Table 5 gives the amount of arsenic in the form of As_2O_3 found in the different organs. Hence, while the amounts of arsenic found in the organs after two to five days treatment were quite variable, showing sometimes the largest amount in the kidney, sometimes in the spleen and sometimes in the liver, in the more chronic cases, the liver contained far more than the other organs both in the entire organ and per

TABLE 4

Arsenic. Arkins and Corper

PREPARATION OF ARSENIC	MILLIGRAMS OF ARSENIC PER GRAM WEIGHT OF TISSUE							
	Normal eye	Tuberculous eye	Blood	Spleen	Testes	Lungs	Kidneys	Liver
Sodium arsenite	0.33	1.0	0.15	1.5		0.666	2.066	5.0
Sodium cacodylate			5.0	6.33	6.266	3.92	5.3	4.0
Atoxyl			15.66	12.52	4.9			6.23
Arsacetin			125.0	20.0		60.0	44.12	17.33
Neosalvarsan			2.63	6.0		12.5	10.0	5.0

TABLE 5

Arsenic

ORGAN	WEIGHT OF ORGAN	AMOUNT OF As_2O_3	MILLIGRAMS OF As_2O_3 PER 100 GRAMS
Liver	1135	184.6	16.0
Spleen	135	1.9	1.4
Lungs	645	19.4	3.0
Kidneys	200	8.5	4.2
Stomach	290	23.8	8.2
Intestines	700	41.6	5.9
Pancreas	80	0.9	1.1
Muscles (thigh)	600	3.3	0.5
Brain	1190	17.4	1.4
Spinal cord	25	0.9	3.6
Uterus and Appendages	85	Trace	Trace
Heart	300	1.1	0.3
Bone (femur)	100	Trace	Trace
Hair	13	Trace	Trace
Total absorbed arsenic			303.4 mgs.
Stomach contents	200	15.8	
Intestinal contents	200	4.0	
Rectal contents	30	7.7	
Total unabsorbed arsenic			27.5 mgs.

hundred grams of tissue. The stomach and intestines occupied second place, the kidneys next and the lungs fourth. It is interesting to note that Underhill found considerable quantities in the brain and spinal cord, the brain containing as much per gram as the spleen and the spinal cord two and a half times as much as the spleen and slightly more than the lungs.

Lead. Blyth reports as follows the distribution of lead in a dog killed by chronic lead poisoning (the amounts given were not stated):

	grams
In bones	0.18–0.27 *mgm. per 1000*
In kidneys	0.17–0.20 *mgm. per 1000*
In liver	0.10–0.33 *mgm. per 1000*
In spinal cord	0.06–0.11 *mgm. per 1000*
In brain	0.04–0.05 *mgm. per 1000*
In muscles	0.02–0.04 *mgm. per 1000*

Traces were found in the intestines, spleen, blood and bile. He also reported a case of a woman to whom lead acetate had been administered frequently during two months. The amount given was unknown, but the poisoner had bought at first 1 ounce and later one-half ounce of the salt. None was administered during the last seventeen days of the patient's life. Lead was found in most of the viscera, 16.2 mgm. being found in the liver, 3.2 mgm. in the brain and 16.2 in one-half of the stomach. Small amounts were present in the spleen, kidneys and lungs. Excretion had taken place by feces and urine. In these two reports, the liver contained more lead than any other organ.

Tin. Salant, Rieger, and Treuthardt found that after subcutaneous injection of soluble tin salts, the metal was excreted in the feces and to a lesser extent in the urine. About 20 to 25 per cent of the metal was found in the skin. The liver contained 1.5 to 8 mgm. or an average of about 5 per cent of the amount injected. Soluble tin salts disappear from the circulating blood in two to three hours. Most of the elimination is through the gastro-intestinal tract and it goes on slowly. Analysis of the other organs and of the rest of the body shows a very general distribution of the tin throughout the body but most of the tin is found in the skin. Salzer recently reported a case of chronic tin poisoning with pronounced nervous symptoms and Schryver reports an experiment in a dog in which 24.5 per cent of the tin administered was recovered from the brain and cord.

Antimony. Glaister reports three cases of chronic poisoning with antimony. The amounts given were unknown. The amounts found were as follows:

	I	II	III
	grams	*grams*	*grams*
Stomach	0.64	0.32	0.08
Bowels and contents	16.64	23.43	1.15
Liver	1.98	4.55	2.42
Kidneys	0.39	0.82	0.18
Brain	0.47		

Blyth states that antimony is eliminated in the urine mostly and only small amounts are found in the organs.

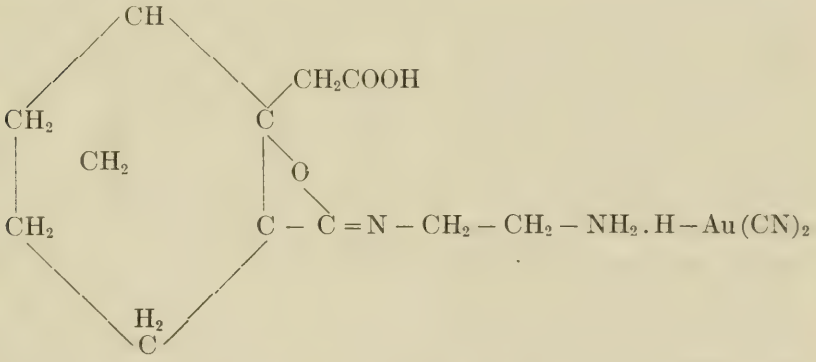
Mercury. Blyth reports analysis of organs and excreta of a dog which received in thirty-one days 2.368 grams of mercury in the form of calomel. About 90 per cent of the entire amount was found in the feces and about 2 per cent in the urine. The liver contained 0.014 grams, the muscles 0.0114 grams and the brain, heart, lungs, spleen, pancreas, kidneys, scrotum and penis together contained only 0.009 grams. Rosenbloom reports the analysis by the Ludwig method of the organs of a woman who died of acute mercuric chloride poisoning. His findings are embodied in table 6.

TABLE 6

ORGAN	MILLIGRAMS Hg. FOUND IN ENTIRE ORGAN	MILLIGRAMS Hg. FOUND PER 100 GRAMS WEIGHT OF ORGAN
Liver	39.82	3.62
Spleen	1.70	1.20
Lungs	Trace	Trace
Kidneys	5.80	2.80
Heart	5.81	1.92
Blood	52.00	1.20
Muscle	25.80	0.06
Stomach	1.50	0.50
Small intestine	4.80	1.60
Large intestine	7.28	1.92
Brain	1.68	0.14
Bile	Trace	Trace
Stomach contents	12.20	
Intestinal contents	8.60	
Rectal contents	8.40	
Total	111.39	

It may be noted from the study of these analyses of various organs for copper, zinc, arsenic, tin, antimony, lead and mercury, that the liver is almost uniformly the organ which contains most of the metal.

In my own work, which has extended over more than two years, small, non-lethal doses of gold salts were introduced into guinea-pigs by intracardiac, subcutaneous or intramuscular injection or by feeding. The pigs were usually fed every day and injected once a week or oftener. The salts used were aurous cyanide (AuCN), auric cyanide ($Au(CN)_3$, Gold potassium cyanide ($AuK(CN)_2$) and cantharidylethylenediamineaurous cyanide, which has the trade name aurocantan. Its formula is given as follows:



This last preparation was made in my laboratory according to the method patented by Spiess and Feldt, since it was impossible to obtain any of the German preparation. The dosage at first was about 2 mgm. but was gradually increased to 5 and later to 10 mgm. The treatment extended over four or five months, and in some cases even longer. During the experiment it became advisable to determine the quantitative distribution of the gold in both normal and tuberculous animals. Since no method of quantitative analysis was known which was delicate enough to determine accurately amounts as small as were to be found in the organs of these animals, a method was developed by my assistant, Sidney M. Cadwell, which will soon be pub-

lished by him in detail. In brief, however, the method is as follows: The tissue is digested in Kjeldahl flasks with concentrated sulphuric and nitric acids. The acid is evaporated down to about 1 cc. and the residue treated with aqua regia to insure the oxidation of the gold. The free chlorine is then removed with ammonia which is neutralized with hydrochloric acid. It is then filtered through asbestos and treated with ammonia and hydrochloric acid to slight acidity. Phosphoric acid and disodium phosphate are added and the solution is made up to 40 cc. and electrolyzed at 60°C. with a voltage of 1 to 1.2 volts. The gold is deposited on carefully cleaned and weighed platinum electrodes. After the time needed for the complete deposition of the gold, the electrodes are cleaned and dried and weighed on a fine assay balance to determine by the difference in weight the amount of gold deposited. By this method it was possible to recover from 80 to 100 per cent of all the gold introduced into the animal and the margin of error was from 0.02 to 0.03 mgm.

Excretion of gold. Several determinations were made of the amount of gold excreted in the urine and feces during the first three to seven days after an injection and in two animals the total excretion during two months of treatment with aurocantan was analyzed. The results of these determinations are given in table 7. In this table it may be seen that from 43 to 55 per cent of the total gold introduced is excreted during the first seven days after an injection, the excretion reaching its highest point on the second and third days. With the exception of no. 1, we find considerably more gold excreted in the urine than in the feces, a point worthy of note, since Blyth reports 90 per cent of the mercury injected excreted in the feces and only 2 per cent excreted in the urine, and both tin and copper were found in the feces in larger amounts than in the urine. In the two pigs in which the urine and feces were saved during the entire two months of treatment, however, the feces contained much more gold than did the urine, which may be accounted for, at least in part, by the fact that the urine was suppressed during a part of the time, the second of the two pigs being anuric during almost the entire time of the treatment. The conclusion

TABLE 7

Per cent of gold excreted

SALT	FIRST DAY		SECOND DAY		THIRD DAY		FOURTH DAY		FOURTH AND FIFTH DAYS		SIXTH AND SEVENTH DAYS		TOTAL FOUND		TOTAL EXCRETED IN TIME TESTED
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	
$AuK(CN)_2$.....	0.0	0.0	0.7	3.3	7.5	8.4							8.2	11.7	19.9
$AuK(CN)_2$.....			17.3	0.9	3.8	2.6	7.8	3.0					28.9	6.5	35.4
$AuK(CN)_2$.....			7.235	7.04									7.235	7.04	14.275
Aurocantan.....			5.4	2.3	6.8	2.3	0.0	0.0					12.2	4.6	16.8
Aurocantan.....	5.5	1.0	9.0	6.5	8.5	0.0			9.0	9.0	7.0	0.0	39.0	16.5	55.5
Aurocantan.....	3.0	2.0	5.0	1.0	9.5	3.0			6.0	2.0	6.0	5.5	29.5	13.5	43.0
Aurocantan.....	16.25	0.0	0.0	0.0	18.7	0.0							34.95	0.0	34.95
Aurocantan.....											9.75	0.0	9.75	0.0	9.75
Aurocantan.....											37.0	10.0	37.0	10.0	47.0
Aurocantan.....													13.85	39.38	53.23 (60
Aurocantan.....													2.78	49.64	52.42 days

TABLE 8

GOLD SALT	TOTAL GOLD GIVEN	DURATION OF TREATMENT	TIME SINCE LAST DOSE	MILLIGRAMS OF GOLD FOUND IN							
				Liver	Spleen	Lungs	Kidneys	L. glands	Injected skin	Intestines	Rest of body
Non-tuberculous animals											
	mgm										
Aurocantan	84	276 days	2 days	2.85	0.31	0 08	0 44	0.0			
Aurocantan	84	275 days	1 day	2.19	0.76	0.16	0.52	0.02			
Aurocantan	14 4	60 days	7 days	0 96	0.26	0.15	0.21		0.25	0.18	3.65
Aurocantan	9 6	75 days	5 days	0.61	0.11	0.04	0.10		0.18		
Aurocantan	4 8	45 days	1 day	0.44	0 07	0.11					
Aurocantan	4 8	43 days	1 day	0.22	0.04	0.03					
Aurocantan	2 2	3 days	1 day	0.07	0.02	0 02	0.06		0.03		
Aurocantan	0 8	12 days	6 days	0.22	0.03	0.067					
$AuK(CN)_2$	254 3	211 days	1 day	1.04	0.12		0.0				
$Au(CN)_3$	192 83	230 days	1 day	1.60	0.05	0.07	0.09				
$AuK(CN)_2$	10 23	3 days	1 day	0.62	0.27	0.15			0.35	0.76	6.696
$AuK(CN)_2$	10 23	6 hours	6 hours	0.79	0.02	0.24	0.11		0.36	0.62	7.597
$AuK(CN)_2$	6 817	4 days	4 days	1.21	0.32	0.16					
$AuK(CN)_2$	6 8	5.5 hours	5.5 hours	0 42	0.03	0.35	0.07				
$AuK(CN)_2$	6 188	1 hour	1 hour	1.03	0.0	0.16					2.15
$AuK(CN)_2$	3 536	40 minutes	40 minutes	0.13	0.0	0.35					2.27

Tuberculous animals

Aurocantan	84	276 days	2 days	1.79	0.91	0.07	0.20	0.07			
Aurocantan	84	279 days	1 day	2.01	2.25	0.10	0.49	0.07			
Aurocantan	31.6	160 days	7 days	1.62	0.22	0.10	0.18	0.02			
Aurocantan	28.0	90 days	7 days	1.09	0.58	0.08	0.53	0.07			
Aurocantan	27.87	236 days	6 days	0.98	0.25	0.14	0.05	0.08			
Aurocantan	17.069	242 days	8 days	0.36	0.16	0.14	0.22	0.14			
Aurocantan	17.065	235 days	8 days	0.24	0.21	0.07		0.06			
Aurocantan	16.80	107 days	3 days	0.32	0.13	0.13	0.11		0.60	0.26	4.71
Aurocantan	15.068	181 days	8 days	0.24		0.10	0.12	0.0			
Aurocantan	14.40	60 days	1 day	0.46	0.13	0.10	0.15		0.30	0.13	4.98
Aurocantan	13.60	56 days	7 days	0.71	0.35	0.11	0.13	0.05			
Aurocantan	13.60	50 days	1 day	0.58	0.35	0.16	0.25	0.10			
Aurocantan	12.26	153 days	6 days	0.95		0.03		0.08			
Aurocantan	11.472	184 days	22 days	0.73	0.15	0.04	0.12	0.06			
Aurocantan	9.6	37 days	1 day	0.73	0.15	0.22	0.27	0.05			
Aurocantan	8.8	115 days	4 days	0.30	0.16	0.08	0.14	0.10			
Aurocantan	5.068	68 days	2 days	0.23	0.00	0.06		0.04			
Aurocantan	4.5	42 days	1 day	0.03	0.09	0.0		0.0			
Aurocantan	2.4	17 days	3 days	0.04	0.0	0.04	0.05				
Aurocantan	1.6	7 days	6 days	0.16	0.05	0.0	0.02	0.0			
Aurocantan	1.07	9 days	0 day	0.0	0.08	0.07					
$Au(CN)_3$	245.58	180 days	1 day	0.54	0.03			0.04			
$Au(CN)_3$	132.48	178 days	1 day	0.81	0.24	0.14	0.14	0.0			
AuCN	129.7	237 days	1 day	0.17	0.02	0.06	0.03				
AuCN	97.94	195 days	1 day	0.36	0.07	0.18	0.09				

TABLE 9

GOLD SALT	TOTAL GOLD GIVEN	DURATION OF TREATMENT	TIME SINCE LAST DOSE	PER CENT OF TOTAL GOLD FOUND IN										TOTAL GOLD FOUND IN ORGANS	TOTAL GOLD FOUND IN BODY
				Liver	Spleen	Lungs	Kidneys	L. glands	Injected skin	Intestines	Blood	Heart	Rest of body		
Non-tuberculous animals															
	mgm.													*per cent*	*per cent*
Aurocantan	84	275 days	1 day	2.607	0.905	0.19	0.619	0.024						4.345	
Aurocantan	84	276 days	2 days	3.392	0.369	0.095	0.524	0.0						4.38	
Aurocantan	14.4	60 days	1 day	6.60	1.81	1.04	1.46		1.74	1.30	0.18	0.0	28.51	10.97	39.48
Aurocantan	9.6	75 days	5 days	6.35	1.10	0.40	1.0		1.75					10.73	
Aurocantan	4.8	45 days	1 day	10.20	1.46	2.29			3.75					13.95	
Aurocantan	4.8	43 days	1 day	4.58	0.625	0.833								6.05	
Aurocantan	2.2	3 days	1 day	3.1	0.9	0.0	2.7		1.5		2.3	2.7		13.2	
Aurocantan	0.8	12 days	6 days	27.5	3.75	7.50								38.75	
$AuK(CN)_2$	254.3	211 days	1 day	0.409	0.047	0.0	0.0							0.456	
$AuK(CN)_2$	192.83	230 days	1 day	0.825	0.026	0.036	0.047							0.934	
$AuK(CN)_2$	10.23	3 days	1 day	6.06	2.64	1.47			3.42	7.46			65.31	10.16	100+
$AuK(CN)_2$	10.23	6 hours	6 hours	7.72	0.2	2.34	1.07		3.52	6.06			74.27	20.91	95.18
$AuK(CN)_2$	6.817	4 days	4 days	17.75	4.69	2.35								24.8	
$AuK(CN)_2$	6.8	5.5 hours	5.5 hours	6.18	2.04	5.15	4.76							18.13	
$AuK(CN)_2$	6.188	1 hour	1 hour	16.6	0.0	2.3						1.2	34.5	20.1	54.6
$Au(CN)_3$ ...	3.536	40 minutes	40 minutes	3.7	0.0	10.0						1.2	63.8	14.9	78.7

Tuberculous animals

Aurocantan....	84	279 days	1 day	2.35	0.383	0.625	0.58	0.08						3.43	
Aurocantan...	31.6	160 days	7 days	5.13	0.70	0.32	0.57	0.06			0.63	0.0		7.41	
Aurocantan....	28.0	90 days	7 days	3.89	2.07	0.29	1.89	0.03						8.17	
Aurocantan....	27.87	230 days	6 days	3.52	0.90	0.50	0.18	0.29		0.82		0.11	10.76	7.18	18.94
Aurocantan....	17.069	242 days	8 days	1.406	1.23	0.410		0.353						3.399	
Aurocantan....	17.065	235 days	8 days	2.109	0.644	0.82	1.288	0.82						5.681	
Aurocantan....	16.80	107 days	3 days	1.905	0.774	0.774	0.655		0.573				22.93	4.346	27.276
Aurocantan....	15.068	181 days	8 days	2.59		0.66	0.78	0.0						4.03	
Aurocantan....	14.40	60 days	1 day	3.19	0.903	0.694	1.04		2.08	0.992	0.0	0.069	34.57	5.896	40.466
Aurocantan....	13.60	56 days	7 days	5.22	2.574	0.809	0.956	0.368				0.0		9.929	
Aurocantan....	13.60	50 days	1 day	4.265	2.574	1.177	1.839	0.734				0.441		11.03	
Aurocantan....	12.26	153 days	6 days	7.74		0.244		0.652						8.636	
Aurocantan....	11.472	183 days	22 days	6.363	1.308	0.349	1.046	0.523						9.589	
Aurocantan....	9.6	37 days	1 day	7.607	1.563	2.292	2.813	0.521				1.402		15.834	
Aurocantan....	8.8	115 days	4 days	3.40	1.75	0.86	1.50	1.15						8.60	
Aurocantan....	5.068	66 days	2 days	4.54	0.0	1.19		0.79						6.62	
Aurocantan....	4.5	42 days	1 days	0.625	1.9	0.0		0.0						1.4	
Aurocantan....	2.4	17 days	4 days	1.669	0.0	1.669	2.084							5.422	
Aurocantan....	1.6	7 days	6 days	10.0	3.13	0.0	1.25	0.0				0.0		14.38	
Aurocantan....	1.07	8 days	0 days	0.0	7.48	6.52								14.0	
$Au(CN)_3$.....	245.58	180 days	1 day	0.19	0.01	0.19		0.02				0.035		0.445	
$Au(CN)_3$	132.48	178 days	1 day	0.8	0.13	0.11	0.07	0.0						1.11	
AuCN.....	129.7	237 days	1 day	0.131	0.015	0.046	0.023							0.215	
AuCN.....	97.94	195 days	1 day	0.368	0.072	0.184	0.092							0.716	

TABLE 10

GOLD SALT	TOTAL GOLD GIVEN	DURATION OF TREATMENT	TIME SINCE LAST DOSE	MILLIGRAMS OF GOLD PER GRAM WEIGHT OF TISSUE IN									
				Liver	Spleen	Lungs	Kidneys	L. Glds.	Injected skin	Intestines	Blood	Heart	Rest of body
Nontuberculous animals													
	mgm.												
Aurocantan	84	275 days	1 day	0.0765	0.4967	0.0276	0.0593	0.0154					
Aurocantan	84	276 days	2 days	0.1379	0.422	0.015	0.063	0.0					
Aurocantan	14.4	60 days	1 day	0.046	0.278	0.028	0.05		0.032	0.013	0.002	0.0	0.012
Aurocantan	9.6	75 days	5 days	0.055	0.27	0.014	0.033		0.034				
Aurocantan	4.8	43 days	1 day	0.005	0.04	0.03							
Aurocantan	4.8	45 days	1 day	0.017	0.093	0.009			0.018				
Aurocantan	2.2	3 days	1 day	0.0036	0.033	0.0	0.013		0.008		0.005	0.04	
Aurocantan	0.8	12 days	6 days	0.011	0.043	0.013							
Average				0.044	0.2094	0.0171	0.0436	0.0077	0.023	0.013	0.0035	0.02	0.012
$AuK(CN)_2$	254.3	211 days	1 day	0.036	0.131	0.0	0.0						
$Au(CN)_3$	192.83	230 days	1 day	0.059	0.088	0.008	0.023						
$AuK(CN)_2$	10.23	3 days	1 day	0.021	0.21	0.027							
$AuK(CN)_2$	10.23	6 hours	6 hours	0.049	0.022	0.048	0.02		0.028	0.013			0.025
$AuK(CN)_2$	6.817	4 days	4 days	0.062	0.421	0.03							
$AuK(CN)_2$	6.8	5.5 hours	5.5 hours	0.022	0.44	0.047	0.015						
Average				0.041	0.219	0.027	0.0145		0.0315	0.013			0.0225

Tuberculous animals

Aurocantan	84	276 days	2 days	0.055	0.474	0.012	0.023	0.0					
Aurocantan	84	279 days	1 day	0.07	0.19	0.019	0.06	0.031					
Aurocantan	31.6	160 days	7 days	0.072	0.18	0.014	0.046	0.012			0.019	0.0	
Aurocantan	28.0	90 days	7 days	0.035	0.053	0.006	0.071	0.040					
Aurocantan	27.87	236 days	6 days	0.038	0.313	0.014	0.009	0.017		0.017		0.006	0.007
Aurocantan	17.069	242 days	8 days	0.012	0.058	0.009		0.019					
Aurocantan	17.065	235 days	8 days	0.0179	0.0409	0.0118	0.0427	0.0921					
Aurocantan	16.80	107 days	3 days	0.016	0.011	0.011	0.020		0.052	0.006		0.015	0.016
Aurocantan	15.068	181 days	8 days	0.016		0.012	0.027	0.0					
Aurocantan	14.40	60 days	1 day	0.017	0.046	0.014	0.034		0.021	0.007	0.0	0.005	0.012
Aurocantan	13.60	56 days	7 days	0.026	0.17	0.01	0.02	0.016				0.0	
Aurocantan	13.60	50 days	1 day	0.019	0.030	0.033	0.012	0.0				0.022	
Aurocantan	12.26	153 days	6 days	0.05		0.015		0.053					
Aurocantan	11.472	184 days	22 days	0.02	0.041	0.003	0.018	0.009					
Aurocantan	9.6	37 days	1 day	0.048	0.043	0.036	0.071	0.025				0.069	
Aurocantan	8.8	115 days	4 days	0.016	0.06	0.012	0.027	0.04					
Aurocantan	5.068	68 days	2 days	0.014	0.0	0.01		0.019					
Aurocantan	4.5	42 days	1 day	0.0063	0.08	0.0		0.0					
Aurocantan	2.4	17 days	3 days	0.002	0.0	0.008	0.017						
Aurocantan	1.6	7 days	6 days	0.016	0.083	0.0	0.007	0.0				0.0	
Aurocantan	1.07	9 days	0 days	0.0	0.08	0.019							
Averages				0.0256	0.098	0.0126	0.0278	0.0303	0.01	0.009	0.0095	0.064	0.0117
$Au(CN)_3$	245.58	180 days	1 day	0.007	0.002			0.01				0.019	
$Au(CN)_3$	132.48	178 days	1 day	0.012	0.017	0.010	0.010	0.0					
AuCN	129.7	237 days	1 day	0.007	0.009	0.016	0.027						
AuCN	97.94	195 days	1 day	0.007	0.009	0.006	0.007						
Averages				0.008	0.009	0.0106	0.015	0.005				0.019	

justified from this table is that in one week after a single injection or during two months of continuous treatment with the gold salts, about one-half of the gold introduced is excreted through the urine and feces. It may be that after several injections the kidneys lose their power to excrete the gold and that then larger quantities are excreted by the gastro-intestinal tract. Table 8 shows the total amount of gold in milligrams found in the different organs of animals dying after certain periods of treatment with the gold salts. The duration of the treatment, the total amount of gold administered and the time since the last dose are given in the table. Table 9 shows the percentage of the total gold used which was found in the different organs. Table 10 gives perhaps the clearest idea of the distribution of the metal, since it indicates not the total amount regardless of the size of the organ, but the amount in milligrams per gram weight of the tissue of the organ. If we compare these two tables with the similar tables of other metals, we are first struck with the important place occupied by the spleen in nearly all cases. As noted before, the liver and kidney, but especially the liver is the main place of deposit of the other metals. In the non-tuberculous animals treated with aurocantan, the spleen contained from three to eight times as much per gram weight of tissue as did the liver and much more than the other organs. The proportion was about the same in the tuberculous animals. In the two pigs in which none was found in the spleen, that organ was small and the total amount of gold in it might well be so small as to be within the margin of error in weighing and analyzing. If we average the thirty cases analyzed we find about four times as much in the spleen as in the liver, while the liver and kidney have about the same average amount per gram, the lungs having somewhat less than the other organs. With the other salts of gold shown in table 10, the differences are not so marked, but the average amount per gram in the spleen is five times that in the liver, which contains more than any other organ. The question naturally arises why should the spleen take up and hold proportionately more gold than other organs and tissues. Since it is known that the spleen is the organ in

which most of the red blood corpuscles are destroyed, it was thought possible that these might be the carriers of the gold to the spleen. To test this point four guinea-pigs received each five subcutaneous injections of cantharidyl-ethylenediamine aurous cyanide. The total amount given was 43 mgm. of the salt or 17.2 mgm. of gold. The animals were killed by bleeding; the blood was received in sodium citrate solution and centrifuged to separate the corpuscles from the serum. These were then analyzed separately for gold. The results are shown in the following table.

TIME AFTER LAST INJECTION	AMOUNT OF BLOOD	GOLD IN SERUM	GOLD IN CORPUSCLES
hours	*cc.*	*mgm.*	*mgm.*
2	3	0.00	0.17
$3\frac{1}{2}$	12	0.00	0.26
$21\frac{1}{2}$	9	0.01	0.00
$22\frac{1}{3}$	9	0.00	0.04

From these analyses, it would appear that the gold in two or three hours after injection is either taken up by the blood corpuscles or is carried down in colloid form with them, since practically none is found in the clear serum. It may also be noted that within 24 hours, the gold has practically disappeared from the blood so that none or a mere trace was found in either serum or corpuscles. It was shown by this experiment and by several others that the gold salt introduced into the subcutaneous tissue is very quickly taken up by the blood. In the blood it is no doubt partly reduced to colloidal gold and partly changed to an albuminate. This gold may cause an unusually rapid destruction of red corpuscles which are taken up and destroyed by the macrophages of the spleen. Much gold might cling to these corpuscles and be carried with them to the spleen. The only probable explanations for the fact that other metals are not thus taken up by the spleen were: (1) that the other metals were held longer and perhaps reduced at the point of injection. (2) As suggested by Dr. Kyes, that the gold salts used i.e., the cyanides, may destroy the red corpuscles more rapidly than do the salts

used of the other metals. Hauck reports a marked reduction in the number of both red and white cells after the use of gold cyanide.

The tuberculous lymph glands varied greatly, occasionally equalling or exceeding the spleen in gold content and averaging more than any other organ except the spleen. The area of skin and subcutaneous tissue surrounding the point of subcutaneous injections was analyzed separately, showing usually about the same amount per gram as the entire animal, indicating that none or very little of the gold injected was retained at the point of injection six hours after the injection. In several cases, after the removal of the organs, the rest of the body, including bones, muscles, etc., was ground and well mixed; aliquot portions of this mixture were analyzed for gold. The amount of gold per gram in the skeleton and skeletal muscles was found to average about 0.01 mg., consistently less than in the important organs. The brain was examined in three cases, but no gold was found. The blood and the heart usually contained mere traces.

The duration of the treatment with the gold salts seems not to exercise any consistent influence on the amount of gold found in the organs. In spite of the fact that only about one-half of the gold introduced seems ever to be excreted, a long time of continuous treatment does not invariably increase materially the concentration of the metal in the organs nor does the time which has elapsed since the last dose affect materially the concentration of the metal.

SUMMARY AND CONCLUSIONS

Gold differs somewhat from other metals in its distribution in the animal body.

Copper, zinc, arsenic, tin, antimony and mercury are generally deposited in the liver much more than in other organs. The kidney may in some cases contain as much and occasionally even more in proportion to its weight than the liver. The spleen usually contains none or mere traces. These metals are excreted for the most part through the gastro-intestinal tract.

Gold seems to select the spleen by preference, since the amount per gram weight or the gold concentration in the spleen is in most cases much greater than in the liver. Sometimes the kidney and sometimes the liver comes second in gold concentration.

About one-half of the gold administered has been recovered from the urine and feces within seven days after a single injection and in two months during which weekly injections were given and all the urine and feces were saved; more is excreted through the urine than through the feces, at least in the earlier part of the treatment.

The gold salts used are readily absorbed after subcutaneous injection, no more remaining in the subcutaneous tissues around the point of injection than in other parts of the body.

Gold salts are quickly taken up from the blood after intracardiac injection and usually no gold or a mere trace is found in the circulating blood twenty-four hours after the injection. Two to four hours after the last injection, no gold was found in the clear serum, although a considerable amount was recovered from the layer of blood cells of the centrifuged blood.

No gold was found in the brain.

The amount of gold administered, the duration of treatment and the time since the last dose seem to exert no constant or consistent influence on either the total or proportional gold content of the animal organs.

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THE USE OF GOLD SALTS IN THE TREATMENT OF EXPERIMENTAL TUBERCULOSIS IN GUINEA-PIGS

XVIII. STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS

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The use of gold in therapy is by no means new and a complete survey of the history and literature has been impracticable. The earliest reference which I have found in the literature states that Abu Moussa Djafar or Geber, surnamed the Wise, who was born at Houran in Mesopotamia in the middle of the 8th century, recommended the use of gold as a cure for every disease of men, animals and plants, asserting that all the metals were diseased except gold.

Sprengel's Arzneikunde (1800) in the chapter on Medical Culture among the Arabians, refers to the many medical and philosophical treatises of Ebn Sina (better known as Avicenna) who lived from 978-1036 A. D. This ancient philosopher recommended gold, silver and other metals for internal use as blood purifiers. Gold foil was also used in his time to coat pills in the belief that the gold coating increased their efficacy.

Naturally the alchemists in their eager interest in gold did not neglect its use in medicine and Paracelsus (1493-1541), the alchemist and chemist who insisted that the true use of chemistry was to make medicines rather than gold, considered gold as a specific in all affections of the heart; he based this belief on the fact that in the mystic scale, gold is in harmony with that viscus. The Paracelsian "Elixir of Life" or panacea for all ills was said to be composed of gold and mercury. The so-called potable gold or liquid gold and its use in medicine dates from this same period and was highly recommended by Roger Bacon and many others. The Sceptical Chymist (Boyle) criticises its use but admits some wonderful cures. In 1540, Lecoque is said to have cured syphilis by the use of gold, which was much like mercury in its action. Hahnemann ascribed great curative power to gold and Burnett recommended trituration of gold leaf 1 : 1,000,000 as a heart tonic.

The use of gold as a cure for alcoholism was long exploited under the name of the Keeley Gold Cure but in 1891 it was confessed that the remedy used contained no gold and that the name was used with fraudulent intent.

In 1917, Marsalongo and Vivaldi reported 32 cases of typhoid and paratyphoid fever successfully treated with colloidal gold. At different times and by different workers, gold has been used in the treatment of syphilis, heart disease, cancer, chronic Bright's disease, typhoid fever, and chronic rheumatism.

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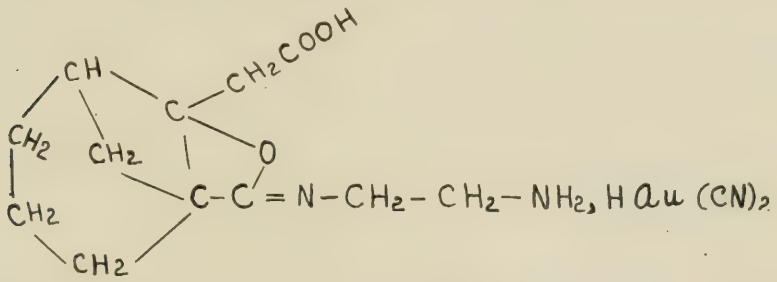
It is also said to have a special influence on the lithemic and fatty degenerations, and on various spinal and cerebral scleroses.

The use of gold in the treatment of tuberculosis, according to White, dates from the time of Paracelsus; for many years after his time, gold in combination with mercury was depended on in the treatment of the cachexias resulting from phthisis, scrofula, syphilis, etc. After a time, however, some serious accidents led to a discontinuance of its use until 1810 when Chrestien brought it back into prominence. In 1890, Robert Koch stated that gold cyanid completely inhibits the growth of tubercle bacilli in dilution of 1:2,000,000 but that it is entirely without influence in the animal body. In 1891, J. B. White recommended the use of double chlorid of sodium and gold combined with iodid of manganese in the treatment of tuberculosis. The effects noted were a distinct prolongation of life in animals, and in human patients gain in weight and appetite, diminution of cough and sputum and râles, absorption of abnormal deposits, and improvement of blood conditions. Merrill also used White's treatment on 20 patients for 2 years and considers its value unquestionable. In 1891 also, the well known Gibbes and Shurley treatment for tuberculosis was published. This consisted of a preliminary iodin treatment for 2 weeks, then double chlorid of gold and sodium for 10 days, then again the iodin, and so on, the 2 treatments alternating. During the time of the treatment, inhalation of chlorin gas was recommended. They reported about 50% of their cases "practically cured."

The next and the most extended group of experiments on the use of gold salts in tuberculosis were published between 1912 and 1915 by a number of German investigators. Several workers reported results from the use of gold potassium cyanid especially in tuberculosis of the skin. Meyer reports good results in general tuberculosis, especially if the gold salt is combined with some substance like borcholin which is said to dissolve the fatty sheath of the tubercle bacillus. Oberstadt reported good influence on the skin lesions, but no case of perfect recovery. He thinks the effect is produced more rapidly if tuberculin treatment is combined with the treatment with the gold salt. Bruck and Glück, after a series of rather unsuccessful experiments in guinea-pigs, used gold potassium cyanid in the treatment of lupus. They ascribed their failure in guinea-pigs to the fact that the gold salt needs to be injected intravenously at frequent intervals and frequent intravenous injections are impracticable in guinea-pigs. Bettmann obtained similar results in the same type of cases. Junker reports good effects from the use of gold salts in pulmonary as well as in skin tuberculosis in patients. Rosenthal injected gold tricyanid directly into the diseased area in men, claiming good results. Poor and Geber also report success from the use of gold potassium cyanid in patients having skin tuberculosis. A series of 7 or more papers, published by Spiess and Feldt, together or separately, between 1912 and 1916, form the most extensive contribution to the literature on the treatment of tuberculosis by means of different salts of gold. These authors consider gold cyanid and gold potassium cyanid too toxic for use in human patients. As cantharidin had earlier been found to have a marked affinity for tuberculous tissues, they conceived the idea of reducing the toxicity of the cantharidin and using it as a carrier for the specific gold cyanid. They were able to reduce the toxicity of the cantharidin 700 times without altering its affinity for tubercles by condensing it with ethylenediamin. This compound was quite nontoxic and combined

readily with gold cyanid. They used first the auric salts but found them 100-1,000 times less efficacious than the aurous salt and therefore in all their later work they used mono-cantharidyl-ethylenediamin-aurous-cyanid, the formula of which they give as follows:



The compound was first tested on about 100 animals during 1½ years and was then used on human patients. They claim that its effects are due partly to a direct inhibitory action on the tubercle bacilli and partly to an indirect action on the defensive agencies of the body. It stimulates connective tissue formation and destroys disease products by its oxidizing power. In experimental tuberculosis in animals, they claim that weight was increased and life was prolonged and tubercles were replaced by scar tissue, especially in rabbits. In guinea-pigs, the effects were not so marked, a fact which they attribute to the necessity of administering the drug subcutaneously. Long continued subcutaneous treatment causes necrosis, and the development of gold tolerance. This was shown in vitro, as, after 1½ months, the bacilli grew on mediums containing 1 part of gold cyanid to 400,000 parts of agar, whereas at first they would not develop in a dilution of 1 part in 2,000,000.

In contrast to the above favorable reports on the use of gold salts in tuberculosis, Koch in 1890 stated that the gold salts were without effect in animals. Perutz and Seppel, Walther, and Schoenfeld admitted that they obtained good results at first, especially in early cases, but stated that these results were transient and did not interfere with the progress of the disease. Blendberger, even in cases of lupus, was unable to obtain results at all comparable to those reported by other workers. Pekanovich, Hauck, and Pasini all call attention to the dangers from the use of gold cyanids, since they are, according to Hauck, severe blood poisons, reducing the number of both red and white cells and causing a severe hemolytic icterus. Pekanovich also reported severe hemorrhages after their use.

On the physiologic and pharmacologic action of gold salts in the animal body, much has been written and it will not be practicable to review more than a few of the most important communications having special reference to the use of gold in tuberculosis. Pereira's materia medica of 1839 states that "its effects are similar to those of mercury. Its salts are corrosive, but less so than mercuric chlorid. In small doses, they are stimulant, promoting the secretion of skin glands, salivary glands and kidneys. In doses of 0.1 gm. in man, they may cause fever, a reaction which Chrestien considered necessary for curative effect. Within proper dosage, no ill effects are produced." Heubner says that the rapidly fatal dose of sodium-gold chlorid is 15 mg. of the salt per kg. of body weight or 7 mg. of gold. If such a dose is given, the blood pressure falls rapidly until it reaches 0 and the animal dies in a few minutes of respiratory

stasis, all the organs showing hyperemia, with occasional ecchymoses and larger hemorrhages. All the veins and capillaries are greatly dilated and often ruptured while the arteries are strongly contracted. In other words, Heubner regards gold salts as vascular poisons. A more chronic intoxication is also described by Heubner. By injecting very slowly and thus avoiding a certain concentration in the blood, more than the acutely fatal dose can be introduced without causing immediate death. In these chronic intoxications, there is no indication of capillary poisoning and no macroscopic change in the organs. Microscopically, the kidney presents the usual picture of metallic poisoning—hyperemia, epithelial degeneration, fat cylinders in the tubules. The lungs show areas of hepatization, and the smaller bronchi are closed by hyaline masses. Many pigmented granular cells are seen. Heubner was unable to find gold microscopically in the organs. Richards, working in Heubner's laboratory, reported finding metallic gold in the nuclei of many organ cells, a report which Heubner failed to corroborate. By chemical analysis, however, Heubner found gold in the blood one half hour after intravenous injection, in the liver, spleen, lungs, and kidneys. Hauck states that gold cyanids cause a great diminution of the erythrocytes and leukocytes in the blood with a severe hemolytic icterus. Junker also notes a rapid lowering of the hemoglobin content of the blood. Bruck and Glück, on the other hand, found no effect on the blood from the use of gold cyanids. Schumacher states that the gold cyanids quickly break down in the body and form probably gold protein compounds which soon release colloidal gold. As the cyanid group is soon synthesized to the nontoxic sulphocyanid or rhodanid, Schumacher thinks that the effects in the body both toxic and therapeutic are due to the colloidal gold and not to the cyanid radicle. Spiess and Feldt and others who are using the gold cyanid in the treatment of tuberculosis admit that hyperemias and hemorrhages result from their use but insist that these are confined to the tuberculous areas, resembling the tuberculin reaction and leading to healing. Colloidal gold solutions have also been injected intravenously. Busquet states that colloidal gold raises the blood pressure and increases the force of the heart beats, diminishing their number, while soluble gold salts have a quite opposite effect, lowering the blood pressure, increasing the frequency of heart beats and diminishing their amplitude.

Koch found that gold in the form of cyanids had very little bactericidal effect on the tubercle bacillus but completely inhibited its growth in a dilution of 1 : 2,000,000. This statement was verified by Rosenthal and by Behring for other bacteria. Behring, however, found that this high inhibitory power in water solutions was reduced in blood serum to 1 : 20,000-30,000. Feldt demonstrated that colloidal gold had an inhibitory effect nearly equal to that of gold cyanid, completely inhibiting the growth of tubercle bacilli in a dilution of 1 : 1,000,000. He also found that gelatin mediums containing 1 part of colloidal gold in 6,000 parts of gelatin remained sterile though left open for 45 days, while control tubes containing no gold were covered in 3 days with a growth of air organisms.

My own experiments on the bactericidal and inhibitory power of gold salts on tubercle bacilli correspond entirely with the findings of

Koch and Behring. DeWitt and Sherman reported that 1 part in 1,000 of gold chlorid is required to kill tubercle bacilli while 1% solutions of gold tricyanid were not always able to kill all the organisms. As opposed to this low bactericidal power, I have found that 1 part in 2,000,000 is able to inhibit completely the growth of these organisms in the test tube. On the other hand, I have found a certain tolerance develop very slowly. After 4 months' growth on agar containing gold, the bacilli would grow very well on mediums containing 1 part of gold in the form of gold potassium cyanid in 2,000,000 and slightly on tubes containing 1 part in 1,000,000. This is not nearly so marked a tolerance as was described by Feldt who states that after 1½ months, he obtained a good growth on dilutions of 1 part of gold cyanid in 400,000 of the mediums. Shiga, on the other hand, asserts that in his experiments the tubercle bacilli developed no tolerance toward gold potassium cyanid. The question of acquired tolerance is one of considerable importance in the chemotherapy of a disease like tuberculosis in which treatment must be continued for long periods. According to our results in the in vitro experiments, however, the tolerance of the tubercle bacillus to gold develops so slowly as not to be a very important factor in the consideration of the treatment of tuberculosis with gold.

The stimulation of the growth of tubercle bacilli by solutions of gold too dilute to kill or inhibit is another important consideration in the use of gold in chemotherapy. We have frequently noticed in our culture tubes that the higher dilutions contained a more luxuriant growth than the control tubes. Takenaka reported that gold salts in broth dilution gave the following results:

1 : 100,000,000 caused stimulation at first.
1 : 10,000,000 caused no inhibition and no stimulation.
1 : 2,000,000 caused no inhibition and no stimulation.
1 : 1,000,000 caused no inhibition but often stimulation.
1 : 500,000 caused no inhibition.
1 : 100,000 caused marked inhibition.

The importance of the consideration of stimulation of growth by a gold concentration below that required to inhibit the growth may easily be appreciated, since it is difficult to keep the concentration of gold in the animal body constantly at or above the point required for inhibition.

My own experiments began Jan. 26, 1915. Eighteen guinea-pigs were inoculated subcutaneously with a strain of human tubercle bacilli of fairly low virulence, the untreated controls dying of generalized

tuberculosis in from 4-10 months. Ten days after inoculation, treatment with gold salts was begun, partly by subcutaneous injection and partly by feeding. Aug. 25, 1915, Sept. 14, 1915, Nov. 17, 1915 and Dec. 6, 1915, and Feb. 28, 1916, other sets were inoculated and treated with gold salts. In most cases, treatment was begun 24 hours after inoculation. In these experiments, some animals were treated with auric cyanid, some with gold potassium cyanid and some with aurous cyanid. All were fed daily and all received injections once or twice a week, sometimes intracardiac and sometimes subcutaneous or intramuscular. The subcutaneous injections often caused ulceration and necrosis of the surrounding tissues; the intramuscular injections of oil emulsions resulted in infiltration of the muscles from the slow absorption, with stiffness, lameness and paralysis. The intracardiac injections were not entirely without danger and a number of animals died immediately after the injection either from hemorrhage or from the toxic effects of the gold cyanids. In many cases, as many as 16 or 18 heart injections were given to the same animals.

Later, desiring to try the effect of the cantharidyl-ethylenediamin-aurous-cyanid recommended by Spiess and Feldt, and being unable to procure any on account of the war, I had some made in my laboratory according to the directions given in the German patent. This preparation was used in the treatment of about 30 tuberculous guinea-pigs. At first it was introduced by intracardiac injection, but later, having found by chemical analysis that it was quickly and completely absorbed from the subcutaneous tissue, the less dangerous method of subcutaneous injection was used. This preparation is much less toxic than the simple cyanids and caused no necrosis in the skin and subcutaneous tissue. Its power of inhibition, however, was somewhat less as the highest dilution which completely inhibited growth of the tubercle bacillus in the test tube was 1 part of gold in the form of aurocantan to 250,000 parts of agar instead of 1 part of gold to 2,000,000 parts of agar.

Having determined that in 1 week from 40-50% of the amount of gold introduced was excreted through the urine and feces, injections were made once a week to keep the concentration in the organism high enough to equal the level necessary for inhibition of the growth of the tubercle bacillus. The dose used at first was usually 2 mg. This was soon increased to 4 mg. and later to 5, then 8, and then 10 mg. of the preparation, about 40% of which is gold. If we could believe that this gold is uniformly distributed throughout the body and retains in the

body an inhibitory power equal to that which it possesses in the test tube, these doses repeated at intervals of 1 week would constantly maintain a concentration sufficient to prevent the growth of any tubercle bacilli in the body. Table 1 will show that this hope was not realized. The total amount of gold administered and the length of life of the animals, as well as the extent of the disease are indicated in the table and are compared with the length of life and extent of the

TABLE 1

A SUMMARY OF THE EFFECT OF TREATMENT WITH GOLD SALTS ON THE PROGRESS OF TUBERCULOSIS IN GUINEA-PIGS

Animals Treated with Gold								
Gold Salt	Mg. Gold	Days of Life	Local Glands	General Glands	Extent of Disease in			Notes
					Liver	Spleen	Lungs	
$AuK(CN)_2$	54.40	64	—	—	++	+++	+++	
$AuK(CN)_2$	92.48	77	+++	—	+++	+++	+++	
$AuK(CN)_2$	92.48	77	+++	+++	+++	+++	+++	
$AuK(CN)_2$	261.80	115	+++	+++	+++	+++	+++	
$AuK(CN)_2$	184.96	120	+++	—	+++	+++	+++	Pericarditis
$AuK(CN)_2$	178.84	125	+++	+++	+++	+++	+++	
$AuK(CN)_2$	189.72	134	+++	+++	+	++	+	
$Au(CN)_3$	145.55	101	+	—	+++	+++	—	
$Au(CN)_3$	211.58	132	+++	+++	+++	+++	+++	
$Au(CN)_3$	209.45	135	+	+	+	+++	+	
$Au(CN)_3$	237.85	150	+	—	+	+++	+++	
$Au(CN)_3$	249.92	160	+++	—	+++	+++	+++	
$Au(CN)_3$	130.64	177	+++	+++	+++	+++	+++	
$Au(CN)_3$	309.56	188	+	—	+++	+++	+++	
$Au(CN)_3$	173,24	211	+++	+++	+++	+++	+++	
AuCN	35.20	44	+	—	—	+	+	
AuCN	35.20	49	+	+	—	+	—	
AuCN	97.68	197	+++	+++	+++	+++	+++	
AuCN	129.36	237	+++	+++	+	+++	+++	
Aurocantan	1.6	7	++	+	—	—	—	
Aurocantan	0.53	9	—	—	—	—	—	Hemorrhage
Aurocantan	2.4	17	—	—	—	—	—	Acute infec.
Aurocantan	9.6	37	+++	+++	+++	+++	+++	
Aurocantan	4.8	42	+	—	—	—	—	Hemorrhage
Aurocantan	13.6	51	+++	++	+++	++	+++	
Aurocantan	13.6	57	+++	+++	+++	+++	+++	
Aurocantan	14.4	60	—	—	—	+++	+++	Killed
Aurocantan	5.06	68	++	+++	—	+	—	Hemorrhage
Aurocantan	28.0	90	+++	+++	+++	+++	+++	
Aurocantan	16.8	107	+	—	—	+++	+++	
Aurocantan	8.5	115	+	+	+	+	—	
Aurocantan	12.26	153	+	+	—	—	—	
Aurocantan	31.6	160	+	+	—	—	—	Hemorrhage
Aurocantan	15.06	181	+	++	—	+++	++	
Aurocantan	11.46	184	+	+++	++	+++	+++	
Aurocantan	90.8	227	+	+	+	+	—	Killed
Aurocantan	90.8	228	++	+++	+++	+	—	Killed
Aurocantan	62.0	232	+	+	+	—	—	Killed
Aurocantan	21.86	236	++	+++	+	+	++	Pneumonia
Aurocantan	17.06	242	+++	+++	+++	+++	+++	Pneumonia
Aurocantan	17.06	247	++	+++	+++	+++	+++	Pneumonia

TABLE 1—*Continued*

A SUMMARY OF THE EFFECT OF TREATMENT WITH GOLD SALTS ON THE PROGRESS OF TUBERCULOSIS IN GUINEA-PIGS

Animals Not Gold Treated (Controls)

Days of Life	Local Glands	General Glands	Extent of Disease in			Notes
			Liver	Spleen	Lungs	
57	+++	+++	+++	+++	+	Pericarditis; pneumonia
57	+++	+++	+++	+++	+	Pericarditis
62	+++	+++	+	++	+++	
92	+++	+++	+++	+++	+++	Pericarditis
97	+++	++	++	++	+	Hemorrhage
98	+++	+++	+++	+++	+++	
100	+++	+++	+++	+++	+++	
105	+++	+++	+++	+	++	Peritonitis
120	+++	—	+++	+++	+++	Pericarditis
122	+++	+	+	+	++	Killed
122	+	+	+++	+++	+++	
133	+++	+++	+++	+++	+++	
143	+++	+++	+++	+++	+++	
147	+++	+++	+++	+++	+++	
167	+++	+++	+++	+++	+++	
174	+++	+++	+++	+++	+++	Tuberculosis of omentum
176	+++	+++	+++	+++	+++	
180	+++	+++	+++	+++	+++	
181	+++	+++	+++	+++	+++	
184	+++	+++	+++	+++	+++	
197	+++	+++	+++	+++	+++	
238	+++	+++	+++	+++	+++	
243	++	—	+++	+++	+++	
264	+++	+++	+	+++	+++	
288	+++	+++	+++	+++	+++	
293	+++	+++	+++	+++	+++	
315	+++	+++	+++	+++	++	
27	+++	+++	—	+++	—	
45	—	—	—	+	+	
59	+++	+++	+++	+++	+++	
104	+++	+++	+++	+++	+++	
105	+++	+++	+++	+++	+++	
105	+	++	+++	+++	+++	
113	+	—	—	—	—	
142	—	—	—	—	—	
162	—	—	—	+++	+++	
206	+++	+++	++	+	—	
226	++	+	—	—	—	Hemorrhage
237	+++	+++	+++	++	—	
285	+++	+	—	+++	+++	
289	+++	++	+++	+++	+	Killed
290	+	—	—	—	—	Killed
289	+	+	—	+	+	Killed
321	+	—	—	—	—	

disease in untreated control animals which were inoculated in the same way and with the same dose of the same strain. In the earlier experiments, the strain of human tubercle bacilli used was of so low a virulence that some of the controls failed to develop the disease or showed only a slight development. Therefore, in later experiments, a much more virulent strain was used. In the table, "local glands" are the lymph glands near the point of inoculation, and the term "general glands" refers to lymph glands in other organs of the body. Absence

of evidence of tuberculosis in an organ is indicated by —, a small number of tubercles is indicated by +, while a very extensive process is shown by +++.

It may be seen from this table that the treatment with gold salts had no beneficial effect on the progress of tuberculosis in guinea-pigs. Life was not prolonged and the extent of the disease in the different organs was certainly not less marked in the treated than in the untreated animals. Indeed, if any distinction can be drawn, it will be in favor of the untreated control animals.

In order to determine whether the gold was well distributed throughout the animal body, my assistants Sidney M. Cadwell and Miss Gladys Leavell developed a method of analysis for the small amounts of gold found in the tissues and analyzed the tissues of a considerable number of animals, tuberculous and nontuberculous, which had been treated with the various gold salts. The details of this analytic work are presented in another paper which will soon be published.* Table 2,† however, presents figures obtained by averaging the amounts obtained in the various organs of all the different animals examined.

TABLE 2

DETAILS OF EXPERIMENTAL WORK TO DETERMINE THE DISTRIBUTION OF GOLD THROUGHOUT THE ANIMAL BODY

Average	$C_4N_4H_4Au$		$AuK(CN)_2$		$Au(CN)_3$		AuCN	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Days lived	130	218.6	101.7	161.5	155.5	161.5	132	161.5
Extent of disease	60%	35%	95%	95%	95%	95%	95%	95%
Mg. gold received	13.34		150.67		210.95		74.36	
Percentage								
gold excreted	40.82		17.55					
gold in organs	9.527		16.4		0.72		0.465	
gold in body	26.13		59.48					
Mg.								
found in liver	0.447		0.69		0.98		0.265	
found in spleen	0.127		0.109		0.083		0.045	
found in lungs	0.071		0.168		0.105		0.12	
found in kidneys	0.12		0.06		0.09		0.03	
found in lymph glands	0.053				0.02		0.09	
found in skin	0.45		0.27					
found in intestines	0.26		0.69					
found in blood	0.069							
found in body	4.08		4.68					
gold per gm.								
of liver	0.0212		0.078		0.027		0.007	
of spleen	0.0916		0.1645		0.0358		0.011	
of lungs	0.0105		0.0305		0.009		0.0085	
of kidneys	0.0263		0.0087		0.0165		0.007	
of lymph glands	0.0246				0.005		0.027	
of heart	0.009							
of blood	0.006							
of skin	0.027		0.0247					
of intestines	0.0075		0.013					
of body	0.012		0.0145					

*Accepted for early publication in the Journal of Pharmacology.
†Reprinted from the American Review of Tuberculosis, 1917, 1, p. 424.

It will be seen from Table 2 that the gold is not uniformly distributed throughout the body, the spleen containing more per gm. weight of the organ than any of the other organs. This may be due to the fact that the spleen is the organ in which red blood cells are for the most part destroyed, and these may easily carry with them an excessive amount of gold. In cases in which the brain was examined, no gold was found in it, and usually none in the blood except for a very short time after injection. If we examine the amount of gold found per gm. weight of the organs, we can readily see that even the smallest concentrations are far greater than the 1 : 2,000,000 generally accepted as the concentration required to inhibit in the test tube the growth of tubercle bacilli, while the amount in the spleen at least is more than sufficient to equal the concentration 1 : 20,000-30,000 stated by Feldt to be sufficient for complete inhibition in blood serum. Yet the spleen is the organ earliest and most severely involved in the tuberculous process. It is difficult to explain the fact that gold salts are so ineffective in the body of the guinea-pig, although found there in concentrations more than sufficient to inhibit growth, unless we assume that in these organs the gold is fixed in the cells and no longer has the power to act on the bacilli.

The main pathologic lesions found in both tuberculous and non-tuberculous animals treated with gold salts are hyperemia of all the organs and fat infiltration of the liver and kidney. Hemorrhages were by no means uncommon, sometimes from the mesenteric vessels and sometimes from thoracic vessels. Frequent small hemorrhagic foci were found in the lungs and occasional hemorrhagic infarcts. In some cases I have noted zones of hemorrhage surrounding tubercles, but I have not found them so common as noted by Spiess and Feldt.

In reports on the efficacy of gold in the treatment of tuberculosis, as indeed is true of all other treatments of tuberculosis, we generally find the statements made that the treatment prolonged life, increased weight, caused scarring of tubercles, etc. Table 1 shows clearly that the duration of life in tuberculous guinea-pigs, whether treated or untreated is very variable, while Table 2 demonstrates that the average duration of life of the controls is greater than that of the treated animals. In any single series of animals, unless a very large dose of a very virulent culture is administered or unless intravenous inoculation is practiced, we will find certain animals living very much longer than the others, even though all received the same dose of the same culture. Thus in one series of guinea-pigs inoculated Jan. 26, 1915, one died May 26, another June 8, another June 23, another July 12, another

July 19, while one lived until Nov. 15, 1915. Lewis noted a similar lack of uniformity in the duration of life in sets of animals receiving the same inoculation. Hence we may say that prolongation of life, unless it is consistent and marked, cannot be taken as an index of the value of any therapeutic agent. As to the weights, it is stated that the controls constantly diminished in weight while the treated animals increased. My experience has been that, unless a massive and overpowering injection is given, practically all animals will increase in weight for a time, beginning to diminish in weight only when the disease has progressed to the point where general intoxication is overpowering the animal and death is imminent. The question of weight, then, depends on dose and virulence of the organism rather than on treatment.

TABLE 3

DAYS OF LIFE AND WEIGHTS OF A SET OF GUINEA-PIGS GIVEN LARGE DOSE OF VIRULENT TUBERCLE BACILLI

	Treated with Aurocantan					Controls				
	1	2	3	4	5	1	2	3	4	5
Days of life	92	51	57	37	7	105	105	27	104	59
Weight, gm.:										
1st week	530	615	525	240	355	580	730	590	295	240
2nd week	520	585	500	230		620	720	650	315	240
5th week	530	615	505	260		625	675	680	335	260
7th week	520	595	510	230		650	630	660	315	240
9th week	520	595	565	230		670	640		355	280
11th week	475	585	555	225		645	645		365	285
13th week	465	585	575			670	645		385	290
15th week	445	580				685	645		403	257
17th week		545				630	510		395	205
21st week		560				635	580		410	
23rd week		525				655	590		430	
25th week		495				650	570		385	
27th week		445				675	570		360	
29th week						610	500			

Table 3 gives the days of life and the weights throughout life of a set of guinea-pigs which received a rather large dose of a virulent culture of human tubercle bacilli. In this set the weights were more irregular and diminished more than with smaller doses of less virulent organisms. Even in this set, the weights, as well as the duration of life were better in the controls than in the treated animals. Furthermore, I have never seen any tendency to connective tissue encapsulation or scarring of tubercles such as is described by many writers.

From my experiments then, the conclusion must be drawn that the gold salts used are not efficacious in the treatment of experimental tuberculosis in guinea-pigs. No attempt has been made to use these salts in human patients and we have no wish to draw any unfavorable conclusions as to the efficacy of gold salts in the treatment of human tuberculosis. But the tendency to hemorrhage and hyperemia in the

gold treated animals and the marked shortening of life on account of the treatment certainly suggest that the treatment of human tuberculous patients with gold is not without danger. Furthermore, the chemical analyses of the organs demonstrate that there is no specific affinity of gold for tuberculous tissues and that the gold in the tissues is probably fixed in the tissue cells in such a form that it cannot inhibit the growth of the tubercle bacillus nor the development of the tubercle even when its concentration in the tissues is much more than sufficient to cause complete inhibition in the test tubes.

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THE EFFECT OF CONTINUOUS INTRAVENOUS INJECTIONS OF ETHYLHYDROCUPREIN ON EXPERIMENTAL PNEUMOCOCCUS INFECTIONS OF RABBITS*

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The invention of the Woodyatt[1] pump for maintaining intravenous injections over protracted lengths of time has led to the suggestion of several lines of research which are being developed in the Sprague Institute. One of these is presented in this article.

The effectiveness of the in vivo action of a therapeutic agent which is used because of its bactericidal properties, in all probability depends on the same factors which operate in the in vitro action of a bactericide, the two chief of which are the time factor and the concentration factor. Either in vitro or in vivo a given bactericide in a stated length of time will act better on bacteria in a higher concentration than in a lower one, and, in a stated concentration, will act better in a long time than in a short time. (It is important to realize that the latter is not infinitely true, since bacteria which are only inhibited or not entirely killed off by a certain low concentration will in time overcome the inhibition and become acclimated to the bactericide and thereupon grow unrestricted and probably with stimulation.) It is impossible to carry out a complete comparison of the in vitro action with the in vivo action of a chemical, because, in the first place, the concentration within the body is limited by toxicity, and in the second place, the concentration is constantly varying, because of (1) chemical destruction of the chemical or neutralization by oxidation or reduction; (2) chemical combination or conjugation with body constituents, and (3) excretion. The time factor, obviously, varies because of the same reasons.

In our usual methods of administering a drug the curve representing the concentration of the substance in the blood at different intervals will be in the shape of a wave. The steepness of the ascent and descent of the wave will depend on the ratio of the rate of absorption and the size of the dose to the above three factors. The length of time which the drug is effective will depend on the shape of the curve and the concentration at which the drug can be effective.

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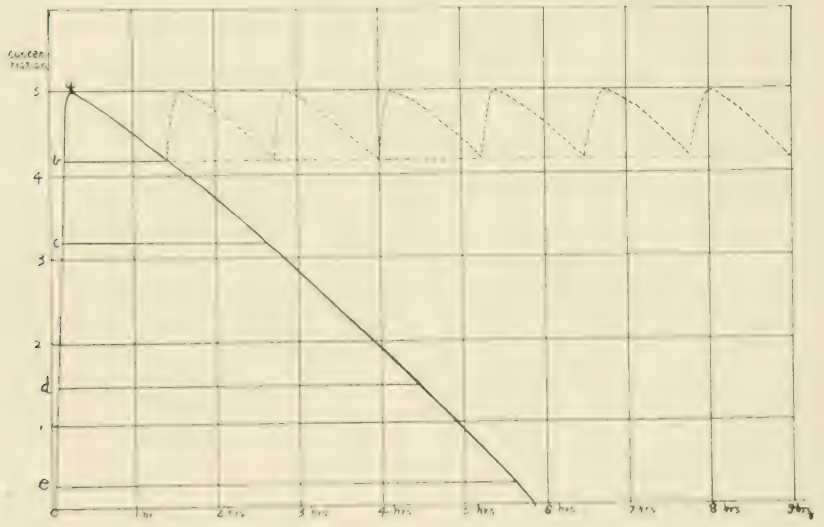
1. Woodyatt: Jour. Biol. Chem., 1917, **39**, 355.

Let the accompanying diagram represent the curve of concentrations which follow the intravenous injection of the largest possible dose of a drug which will not cause toxic symptoms. At once the concentration in the blood rises from zero to the point *a*. Let it be supposed that the three factors mentioned operate so that the concentration decreases at the rate represented by the descending portion of the curve. If the concentration at which the drug is active is at *a*, it is effective only a very short time, but if it is active at *b* it will be effective much longer, and so on, the time of effective action depending on the height of the wave, the rate of its decline, and the concentration of activity. An ideal drug can be pictured in this way showing that such a drug could obtain a high concentration (because of its non-toxicity), would be slow in decreasing this concentration, and would



be highly active at a low concentration. According to the curve, if the drug was active at the concentration *b*, there are two ways in which the time of action could be lengthened: one, by repeating the original dose, or fractions thereof, whenever the concentration fell to *b*. Schematically the concentration in the blood would then be represented by a series of small waves. The second way to maintain an effective concentration would be to introduce the drug *continuously* at a rate which would strike an equilibrium with the rate of operation of the factors which decrease the concentration. In this way the concentration would be schematically represented by a straight line. Such a process as this is now possible since the invention by Woodyatt[1] of a machine which is capable of delivering into the blood stream an accurate amount of fluid at a constant rate.

Let us compare these two suggested methods, first, with the more usual method of medication, and then with each other. While the maintenance of an effective concentration would enhance the action of a drug on bacteria, there would be an increased toxic action on the body tissues. This would, of course, be more marked the nearer the effective concentration approached the toxic concentration. The result would be either a fatal intoxication or a paralysis of the auxiliary forces which operate in the recovery from infections. In the latter case, unless the drug produces a *sterilisans magna*, the unkilled bacteria would be able to grow unrestricted in an almost inert organism. However, if the procedure should kill off all the bacteria, its use would be justified in spite of some toxic action. This is just exactly what does occur in some of our most important forms of medication; for example, in the treatment of syphilis, in which it is the usual procedure to obtain mercurialism and iodism in order to completely kill off the spirochetes. When the concentration is allowed to drop below effectiveness it is possible that in the intervals the bacteria will acquire a "drug resistance" which renders further treatment less effective. On the other hand, in giving frequently repeated doses to maintain an effective concentration, the toxic effect is apt to be more than in the maintenance of a constant concentration which is just effective, because, as represented schematically in the diagram, the concentration occurs in the form of small waves, the crests of which are above the effective concentration but which approach nearer the toxic concentration. In the continuous method a concentration that is above the effective one can be avoided.

It is purposed to follow up this line of research which is opened by the invention of the Woodyatt pump. The plan will be to study the curve of effective concentration in the blood stream, when various drugs are injected intravenously at a continuous and constant rate, on the various physiologic processes, and to put the method to actual test in treating disease conditions.

For various reasons the first drug the study of which has been undertaken in this manner is ethylhydrocuprein (optochin).

After the announcement by Morgenroth and Levy[2] of the discovery and properties of ethylhydrocuprein it was believed that this substance would play an important part in the therapy of pneumococcus infections in man. Quite contrary to these expectations, however, many reports subsequently appeared on the results obtained in its use in these infections, the general trend of which was that in pneumonia the effect of the drug was doubtful, not at all comparable to its in vitro action and to the striking cures obtained by Morgenroth in mice

2. Morganroth and Levy: Berl. klin. Wchnschr., 1911, **48,** 1560 and 1679.

infected with the pneumococcus. In the treatment of exposed pneumococcus infections, as corneal ulcers, where local applications can be made, the drug is of considerable value. As to its value in other local infections, as pleuritis, peritonitis, arthritis, and meningitis caused by the pneumococcus, where direct applications of solutions of ethylhydrocuprein can be made, there seems to be no information.

The studies of Moore[3] present data which give the best information why ethylhydrocuprein is inactive in treating pneumonia. He demonstrated that the drug is slow in action, has small powers of penetration, quickly disappears from the circulation, and has a toxic dose very close to its active dose. For these very reasons, as pointed out in the foregoing, a possible advantage may be found in the continuous injection of the drug. Moore himself anticipated this idea and adopted frequently repeated dosages in his treatments in human infections, but because of the theoretical advantages of the continuous injections over frequently repeated dosage we have not deviated from our purpose, in spite of his negative results.

TECHNIC AND METHODS

Rabbits were chosen as the experimental animal because they are most suitable for the method. In the first place, the easily accessible ear veins make repeated protracted injections possible and easy. Again, the septicemia produced in rabbits by the pneumococcus makes an ideal condition for the in vivo testing of a bactericide. Further, the experimental work that has been done on rabbits with ethylhydrocuprein, in which single dosages were given, has been with negative results.[4] If any beneficial results are obtained they may be justly attributed to the method used.

The descriptions of the Woodyatt pump and its operation have been sufficiently made and a repetition will be omitted here.

The first procedure was to find out the toxicity of ethylhydrocuprein when given intravenously for protracted periods at constant rates. Amounts of the drug were weighed out and dissolved in an amount of 0.9 per cent. sodium chlorid solution, so that when injected at the rate of from 18 to 30 c.c. per hour a certain number of milligrams per kilogram of body weight was delivered. The water used for making the salt solution was redistilled from glass and the sodium chlorid was Merck's fused product. The solution was filtered twice through hard filter paper and sterilized. The syringe of the injecting machine, the buret, rubber tubing and needle were sterilized before

3. Moore and Chesney: The Archives Int. Med., 1917, **19,** 611.

4. Scott: Jour. Path. and Bacteriol., 1914-1915, **19,** 130. Boecker: Ztschr. f. Immunitätsforsch. (Orig.), 1915, **24,** 148.

using. As it passed into the vein the fluid was warmed in a bath of warm water. In determining the toxicity, periods of six hours of injection were used and 2, 5, 10, and 15 mg. per kilogram of body weight per hour were given. No symptoms of toxicity were obtained until 15 mg. per kilogram per hour were given. The effect of this amount was inconstant. This amount could be given at times safely for ten hours. At other times death occurred during the first one or two hours of injection. No factor was found which was responsible for this variance. On the other hand, 10 mg. per kilogram per hour could be given indefinitely without apparent toxic symptoms (ten-hour periods on three successive days), although in one instance sudden death occurred during the first hour of a second period of six hours each.

The effect on the bactericidal action of the serum for these rates was studied. Before the injection was started a sample of blood was taken from the heart. After three hours of injection another sample was taken, and another at the end of the injection. The serum from each sample was put in the ice box and next morning they were heated to 56 C. for one-half hour. To equal amounts of each sample 0.5 c.c. of a 1 : 1,000,000 dilution of a twenty-four-hour broth culture of pneumococcus was added. At various intervals 0.1 c.c. was removed and plated with 10 c.c. of blood agar. At the end of twenty-four hours the colonies on these plates were counted.

There was no effect on the bactericidal action of rabbit serum when ethylhydrocuprein was injected at a rate under 10 mg. per kilogram-hour (Table 1). At this rate, however, there was a slight bactericidal effect, most pronounced at the end of injection. When given at 15 mg. per kilogram-hour there was a rather marked bactericidal effect, most evident, also, at the end of injection (Table 2). This is the first time that it has been shown that ethylhydrocuprein can reach a bactericidal concentration in rabbit's serum, as other workers[5] did not produce it when using single and repeated doses. It has been shown that the failure to obtain such a result with the drug is due to its rapid destruction in the liver (Scott[4]). We surmise that in the continuous injections the power of the liver to destroy or combine ethylhydrocuprein is either fatigued or that the rate of injection exceeds the rate at which the liver can act.

The next procedure was to find the influence of the continuous injection on infections produced in rabbits with the pneumococcus. A twenty-four-hour culture in serum broth of Type 1 was used as the infecting organism. Various intervals of time occurred between experi-

5. Wright: On Pharmacotherapy and Preventive Inoculation Applied to Pneumonia, 1915.

ments and the virulence of the organism varied, but each time the minimum lethal dose[6] (M. L. D.) was determined and the proper controls were made either with the same culture at the time of the ethylhydrocuprein experiment or the day before or after. The infecting dose was given intravenously and was immediately followed by treatment. At the end of each treatment a smear and culture were made from an ear vein, and on death a necropsy was held and cultures made of the heart's blood. The details[7] are given in Table 3.

TABLE 1.—Effect on Bactericidal Action of Rabbit Serum of Giving Ethylhydrocuprein Intravenously at the Rate of 10 Mg. per Kilogram Hour

	After 15 Minutes	After 45 Minutes	After 2 Hours	After 4 Hours	After 7 Hours	After 24 Hours
Normal serum	34	..	46	418		
Three hours of injection	44	18	28	31	24	
Seven hours of injection	37	25	19	44	38	

TABLE 2.—Effect on Bactericidal Action of Rabbit Serum of Giving Ethylhydrocuprein Intravenously at the Rate of 15 Mg. per Kilogram Hour

	Immediately	After 30 Minutes	After 1 Hour	After 2 Hours	After 4 Hours	After 6 Hours	After 24 Hours
Normal serum	15	16	11	18	200	1,000	
Three hours of injection	14	16	18	10	18	46	
Seven hours of injection	19	17	6	6	3	0	0

The results of the experiments can be summarized shortly by saying that in no case was any beneficial effect on the infected animals observed, and in some cases the treated animals died sooner than the controls.

Two modifications of the routine method of treatment were made in an effort to eliminate certain factors which may have been responsible for the negative results. When 21 c.c. are given each hour intravenously, at the end of ten hours there has been injected in all 210 c.c. If no urine, or only a small amount, is excreted in ten hours, the volume

6. A minimum lethal dose is one that will kill within twenty-four hours. If a rabbit overcomes an infection for from thirty-six to forty-eight hours it is not likely to die at all, and in order to be sure of a fatal dose the twenty-four-hour period was selected.

7. At the end of each injection blood cultures were made from an ear vein, and at death smears and cultures were made from the heart's blood; in every case these were positive, and in the last experiment a count of the bacteria showed four times as many in the blood as with the control.

of blood had practically been doubled, which, besides decreasing the concentration of ethylhydrocuprein also decreases the concentration of antibodies. To obviate this the drug was given in a 2 per cent. sodium chlorid solution, which is markedly diuretic. Nevertheless, no improvement in the results was obtained.

Another modification was to give a large initial dose at once and to follow this immediately with periods of continuous injections of 15 mg. per kilogram-hour, of three hours each. The results differed in no way from the others.

TABLE 3.—Results of Treating Rabbits Infected with Pneumococcus with Ethylhydrocuprein Injected Intravenously *

Weight of Rabbit, Gm.	Amount of Optochin per Kilo-Hour, Gm.	Amount of NaCl Solution per Hour, C.c.	Hours of Injection	Dose of Pneumomoccus	Results of Treatment
1,670	0.0082	18	7	1-24 hour slant (5 M. L. D.)	Dead within 24 hours
1,625	0.0074	18	7	1-24 hour slant (5 M. L. D.)	Dead within 24 hours
2,187	0.010	18	First period, 6 hours; second period (after 3¼ hours' interval) 4 hours	2 c.c. of broth culture (5 M. L. D.)	Dead within 24 hours
1,830	0.011	21	First period, 6 hours; next morning, 6 hours	1 c.c. of broth culture (2 M. L. D.)	Died suddenly at the close of second injection
1,680	0.01	21	6	0.5 c.c. of broth culture (5 M. L. D.)	Died within 24 hours
1,700	0.01	21	6 hours after 3 hour interval, 1 hour	0.1 c.c. of broth culture (10 M. L. D.)	Died during injection
1,747	0.01	21	8	0.001 c.c. of broth culture (1 M. L. D.)	Died within 24 hours

* This protocol lists only typical experiments and does not include all experiments done.

While these results do not bear out the theory at the basis of continuous intravenous injections, it is probably because of the nature of the drug and not because of the method. These results lead to the same conclusions as those of Moore, that the drug is useless in the routine treatment of pneumonia.

It is possible that a study of the action of ethylhydrocuprein given continuously in this way on the action of antibodies may explain in some way the paradoxical fact that the pneumococcus septicemia progresses in spite of a demonstrable bactericidal action of the serum.

SUMMARY

1. Ethylhydrocuprein can be given to rabbits intravenously and continuously over protracted lengths of time at the rate of 10 mg. per kilogram per hour, without apparent toxic effects.

2. When injected intravenously into rabbits at the rate of 10 mg. per kilogram per hour for seven hours, ethylhydrocuprein produces

a slight bactericidal action of the serum. A much higher bactericidal action is produced when it is injected at the rate of 15 mg. per kilogram per hour for seven hours.

3. The effect of a fatal dose of pneumococcus on rabbits is not affected by the continuous intravenous injection of ethylhydrocuprein, in spite of the fact that the animal's blood may be distinctly bactericidal in vitro.

4. The continuous intravenous injection of a soluble bactericidal therapeutic drug has theoretical advantages over the usual single or intermittent dosage. The failure of the method with ethylhydrocuprein is probably due to the nature of the drug and not to the method.

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THE METABOLISM OF GLYCINE GIVEN INTRAVENOUSLY AT CONSTANT RATES.

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For a long time the progress of our knowledge of the fate of ingested protein was halted because of the inability to trace it further than the lumen of the intestines. We were certain that it was digested by enzymes more or less completely to the amino-acid stage, but further than this we were unable to go because of the lack of methods for detecting amino-acids in the blood or of identifying possible products of the ingested protein after leaving the intestines. As a result there were formulated a number of hypotheses to explain the method by which the body received into its substance the protein of the food. All these hypotheses had arguments in their favor but each lacked decisive data which proved it. It was not until Van Slyke devised a method for determining small amounts of α-amino-acids that we were fairly certain that proteins were absorbed into the blood stream as amino-acids. By this method the distribution and fate of these absorbed amino-acids can be followed. It is shown that they are quickly removed from the blood stream by the tissues, especially the liver and muscles, and in these tissues further steps in their metabolism are carried on. The first of these steps appears to be the splitting off of the NH_2 from the carbon moiety, the process of deaminization. Van Slyke shows that this occurs at a much slower rate than that at which the amino-acids are removed by the tissues from the blood stream. The NH_2 group is eliminated in the urine as urea and ammonia. The deaminized portion ultimately is oxidized to CO_2 and water. A certain part of the amino-acid is no doubt used for the synthesis of new body proteins.

Since we are now fairly well acquainted with the qualitative history of the amino-acids, refinements of study can be introduced which involve a quantitative aspect. Certain viewpoints which have been developed in the study of carbohydrate metabolism can now be applied to amino-acids. The most recent idea is that of Woodyatt who introduces the factor of rate in the study of intermediate sugar metabolism in the attempt to interpret the biological processes concerned in terms of quantitative chemistry. His method is to introduce sugar or other soluble metabolites into the circulation at known and constant rates for considerable periods of time until equilibria are reached. A study of the rates of excretion and other indices of metabolic processes under these conditions will give data concerning the amount and manner of utilization which can be discussed from the view-point of reaction velocities. In order to maintain a constant rate of entry into the blood stream and to avoid the uncertainties of absorption times, Woodyatt devised an electrically driven machine which delivers measured amounts of fluid directly into the blood stream, accurately adjusted at so much per unit time. With this apparatus, intravenous injections can be kept up indefinitely and constantly at any desired rate. For a fuller discussion of the theory and method of timed intravenous injections the reader is referred to Woodyatt's articles.[1]

This paper is the first of a series in which the metabolism of various amino-acids will be studied in the same manner as Woodyatt *et al.* have studied sugars. They found in the case of glucose that this sugar can be injected for at least 9 hours at any rate up to and including 0.85 gm. per kilo of body weight per hour without causing glycosuria. Above this rate glucose regularly appears in the urine. The question which concerns us now is how rapidly any given amino-acid can be injected continuously without producing an overflow of unchanged amino-acid in the urine.

[1] Woodyatt, R. T., Studies on intermediate carbohydrate metabolism. *Harvey Lectures*, 1915-16, xi, 326; The method of timed intravenous injections. *J. Biol. Chem.*, 1917, xxix, 355. Woodyatt, R. T., and Sansum, W. D., Studies on the theory of diabetes. VIII. Timed intravenous injection of glucose at lower rate. *Ibid.*, 1917, xxx, 155.

The amino-acid selected for study first is glycine, because it is the simplest amino-acid and is easily prepared in pure form. The sample used was prepared by Dr. E. J. Witzemann of this Institute by synthesis from monochloroacetic acid and ammonia. The product was of a high degree of purity, occurring as large colorless rhombic crystals (melting point 243–245°, corrected). The glycine was dissolved in 0.9 per cent sodium chloride solution at approximately the desired concentration. The exact concentration was determined by the Van Slyke amino-acid method. Dogs which had fasted for 24 hours were used. Before each experiment began, the bladder was washed out. There was always a period of 2 hours or more preceding the injection during which a sample of normal urine was obtained. In some of the experiments salt solution was injected before the glycine solution, in order to insure the hourly secretion of enough urine for analysis. Glycine was injected at rates varying from 0.1 to 1.5 gm. per kilo of body weight per hour. On all samples the following determinations were made: total nitrogen by the Kjeldahl method, ammonia nitrogen by the Folin aeration method, urea nitrogen by the Marshall urease method, and amino-acid nitrogen by the Van Slyke method. Comparisons of the effect of the injection of glycine were made with the basal excretion of nitrogen determined from the analysis of the urine of the fore period. Owing to the limitations of the method for determining amino-acid in the urine, together with the small quantities of urine excreted per hour by dogs weighing from 7 to 16 kilos under the conditions of the present experiments, it was not found feasible, except in certain cases, to follow accurately the hourly excretion of the several nitrogenous substances named above. The urine was therefore collected for periods of 2 to 6 hours or more. In the case of the more rapid glycine injections, toxic effects prohibited their being sustained for more than an hour. Accordingly equilibria as indicated by the attainment of constant ratios between the rate of nitrogen injection and the rate of nitrogen excretion were not established except in the case of the lower injection rates. But the short injections at the higher rates show roughly the relative quantities of nitrogen excreted in the form of unchanged glycine and as urea and ammonia, respectively, following injections at these definite rates.

It appears that the tolerance limit for glycine injected into the systemic venous blood of resting dogs is close to 0.2 gm. per kilo per hour. More experiments would be necessary to fix this value; *e.g.*, repeated experiments at 0.3, 0.2, and 0.1 gm. rates. But it is suggested that glycine entering the systemic blood stream at a rate not exceeding 0.2 gm. per kilo per hour may enter the tissues and be metabolized as fast as it is injected, hour after hour, while with faster rates of injection the metabolism of glycine fails to keep pace with the rate of supply and some glycine passes through the body unchanged. When this figure is compared with the tolerance limit for glucose (0.85 gm. per kilo per hour) it forms a basis for comparing the relative normal velocities of the glucose and glycine metabolism.

The data also show, in harmony with established facts concerning amino-acids in general, that their NH_2 groups are split off and enter into formation of urea even when the amino-acids are injected directly into the systemic blood stream. The excretion of this urea begins soon after injection but may drag along for 18 hours or more afterwards. The excretion of unchanged glycine, on the contrary, appears to be completed at a much more rapid rate, although far more slowly than that of sugar. In the latter case we are dealing with an excretion rate; in the former, the rate of metabolism and the rate of excretion both come into play. This suggests the possibility of measuring the velocity of deaminization by determining the rate of urea excretion after simple urea injection and after injection of amino-acids (subtracting one from the other).

Lusk[2] and others found that glycine is converted into glucose in the completely diabetic organism in the ratio of 0.8 gm. of glucose from 1 gm. of glycine. From this fact it has been held that the carbon residue of glycine after deaminization undergoes the same changes in the normal body. If glycine is thus converted into glucose in the normal non-diabetic body, and this conversion occurs at an absolute rate above 0.85 gm. of glucose per kilo of body weight per hour, it might be expected that a glycosuria would occur. It would require the metabolism of 1.06

[2] Lusk, G., Elements of the science of nutrition. Philadelphia, 3rd edition, 1917.

gm. of glycine per kilo of body weight per hour to introduce glucose into the metabolism at the rate of 0.85 gm. per kilo per hour on the basis of Lusk's conception. In the present experiments, glycosuria was regularly absent, but in none of them was there any evidence that the rate of glycine metabolism equalled 1.06 gm. per kilo per hour. These particular experiments therefore cast no light on this subject. At the highest injection rate, 1.5 gm. for 1 hour, we do not know how the material was metabolized and with the long slow injection we would not expect glycosuria. This question might be attacked successfully by a study of the rate of sugar excretion in the completely diabetic animal when glycine was injected at various rates.

It was interesting to note that in Experiment V, during the 3rd hour of injection the dog became very ill. It vomited, had diarrhea, and suffered from severe convulsions, very much resembling the symptoms one sees in Eck fistula dogs after feeding large amounts of meat. Just as soon as the injection was discontinued these symptoms disappeared.

SUMMARY.

1. The tolerance rate for resting dogs of glycine injected continuously into a systemic vein is probably close to 0.2 gm. per kilo of body weight per hour.

2. Glycine thus injected intravenously at constant rates is readily converted into urea.

3. The excretion of unchanged glycine is materially slower than that of sugars.

Experiment I.

Injection of Glycine at the Rate of 1.5 Gm. per Kilo of Body Weight per Hr. for 1 Hr.

Weight of dog, 7.22 kg. Injected 10.83 gm. of glycine, containing 2.03 gm. of N.

Period I = 6 hrs. Normal control. Before injection.
" II = 6 " Period during which glycine was injected for 1 hr.
" III = 12 " After period with no injection.

Analysis of Urine.

Period.	Hrs.	Total N.	Urea N.	Ammonia N.	Amino N
		gm.	*gm.*	*gm.*	*gm.*
I	6	0.476	0.34	0.04	—
II	6	1.800	1.04	0.03	0.428
III	12	1.748	1.35	0.04	0.039

Amount of N injected as glycine, *gm*	8.03
" " N recovered " " *gm*	0.47
Injected glycine excreted unchanged, *per cent*	23.1
Amount of extra N in urine resulting from injection (total), *gm*	2.12
Injected N recovered in urine, *per cent*	100

It would appear that the excretion of glycine had almost or quite come to an end and that the excretion of urea had reached the basal level at the close of the After Period III, while the ammonia excretion remained unaffected by the injection.

Experiment II.

Injection of Glycine at the Rate of 1.13 Gm. per Kilo of Body Weight per Hr. for 1 Hr.

Weight of dog, 12.5 kg. Injected 14.13 gm. of glycine, containing 2.64 gm. of N.

Period I = 6 hrs. Normal control. Before injection.
" II = 6 " Period during which glycine was injected for 1 hr.
" III = 12 " After period with no injection.

Analysis of Urine.

Period.	Hrs	Total N.	Urea N.	Ammonia N.	Amino N.
		gm.	*gm.*	*gm.*	*gm.*
I	6	1.166	0.87	0.075	0.021
II	6	2.254	1.318	0.082	0.276
III	12	2.564	1.758	0.122	0.033

Amount of N injected as glycine, *gm*	2.64
" " N recovered " " *gm*	0.25
Injected glycine excreted unchanged, *per cent*	9.5
Amount of extra N in urine resulting from injection (total), *gm*	1.32
Injected N recovered in urine, per cent	50.0

It appears that the excretion of glycine ended during Period II when the urea excretion probably continued high through Period III. The

ammonia excretion was not changed significantly. The percentage of injected glycine which escaped in the urine is one-half of that in Experiment I.

Experiment III.

Injection of Glycine at the Rate of 1 Gm. per Kilo of Body Weight per Hr. for 2 Hrs.

Weight of dog, 16.27 kg. Injected 32.54 gm. of glycine, containing 6.08 gm. of N.

Period I = 3 hrs. Normal control. Before injection.
" II = 2 " Period during which glycine was injected for 2 hrs.
" III = 2 " After period. No injection.

Analysis of Urine.

Period.	Hrs.	Total N.	Urea N.	Ammonia N.	Amino N.
		gm.	*gm*	*gm.*	*gm.*
I	1, 2, 3	0.468	0.274	0.C2	0.039
II	4	0.670	0.253	0.035	0.269
	5	0.834	0.257	0.038	0.478
III	6, 7	1.003	0.621	0.062	0.089

Amount of N injected as glycine, *gm.*	6.08
" " N recovered " " *gm.*	0.78
Injected glycine excreted unchanged, *per cent*	C2.8
Amount of extra N in urine resulting from injection (total), *gm.*	1.88
Injected N recovered in urine, *per cent*	30.9

The experiment does not permit the decision that the excretion of unchanged glycine was ended when the experiment closed. It appears to have been still in progress 2 hours after the injection. At this time the urea excretion was still rising.

Experiment IV.

Injection of Glycine at the Rate of 0.75 Gm. per Kilo of Body Weight per Hr. for 1 Hr.

Weight of dog, 11.25 kg. Injected 8.44 gm. of glycine, containing 1.58 gm. of N.

Period I = 6 hrs. Normal control. Before injection.
" II = 6 " Period during which glycine was injected for 1 hr.
" III = 12 " After period. No injection.

Analysis of Urine.

Period.	Hrs.	Total N.	Urea N.	Ammonia N.	Amino N.
		gm.	*gm.*	*gm.*	*gm.*
I	6	0.508	0.380	0.022	0.007
II	6	1.076	0.841	0.055	0.070
III	12	1.527	1.289	0.028	0.080

Amount of N injected as glycine, *gm*..... 1.58
" " N recovered " " *gm*..... 0.13
Injected glycine excreted unchanged, *per cent*..... 8.23
Amount of extra N in urine resulting from injection (total), *gm*..... 1.08
Injected N recovered in urine, *per cent*..... 68.3

The glycine and urea excretions were apparently still in progress at the end of Period III.

Experiment V.

Injection of Glycine at the Rate of 0.5 Gm. per Kilo of Body Weight per Hr. for 3 Hrs.

Weight of dog, 15.22 kg. Injected 22.83 gm. of glycine, containing 4.28 gm. of N.

Period I = 3 hrs. Normal control. Before injection.
" II = 3 " Period during which glycine was injected.
" III = 3 " After period. No injection.

Analysis of Urine.

Period.	Hrs.	Total N.	Urea N.	Ammonia N.	Amino N.
		gm.	*gm.*	*gm.*	*gm.*
I	1, 2, 3	0.491	0.421	0.02	0.026
II	4	0.503	0.394	0.019	0.012
	5	0.873	0.738	0.02	0.026
	6	1.029	0.745	0.029	0.066
III	7, 8, 9	2.167	1.738	0.061	0.047

Amount of N injected as glycine, *gm*..... 4.28
" " N recovered " " *gm*..... 0.10
Injected glycine excreted unchanged, per cent..... 2.33
Amount of extra N in urine resulting from injection (total), *gm*..... 3.1
Injected N recovered in urine, *per cent*..... 72.43

The excess of amino N excretion had ceased at the close of Period III. The urea N had decreased but was not as yet equal to basal excretion. No significant change in ammonia N.

Experiment VI.

Injection of Glycine at the Rate of 0.2 Gm. per Kilo of Body Weight per Hr. for 7 Hrs.

Weight of dog, 14.75 kg. Injected 20.65 gm. of glycine, containing 3.86 gm. of N.

Period I = 2 hrs. Normal control. Before injection.
" II = 7 " Period during which glycine was injected.

Analysis of Urine.

Period.	Hrs.	Total N.	Urea N.	Ammonia N.	Amino N.
		gm.	*gm.*	*gm.*	*gm.*
I	1, 2	0.299	0.169	0.014	0.03
II	3	0.305	0.151	0.015	0.023
	4	0.342	0.208	0.018	0.02
	5	0.431	0.299	0.01	0.015
	6	0.555	0.389	0.014	0.014
	7	0.474	0.344	0.008	0.022
	8	0.599	0.455	0.016	0.016
	9	0.573	0.417	0.02	0.017

Amount of N injected as glycine, *gm.*	3.86
" " N recovered " " *gm.*	0.022
Injected glycine excreted unchanged, *per cent.*	0.57
Amount of extra N in urine resulting from injection (total), *gm.*	2.23
Injected N recovered in urine, *per cent.*	57.8

It looks here as if the 0.2 gm. rate barely overtopped the tolerance limit if it did at all. The excess amino N in urine in 3rd and 4th hours is not greater than the limits of error in the methods employed. After that there was no excess whatever.

Experiment VII.

Injection of Glycine at the Rate of 0.1 Gm. per Kilo of Body Weight per Hr. for 7 Hrs.

Weight of dog, 9.08 kg. Injected 6.36 gm. of glycine, containing 1.19 gm. of N.

Period I = 2 hrs. Normal control. Before injection.
" II = 7 " Period during which glycine was injected.

Analysis of Urine.

Period.	Hrs.	Total N.	Urea N.	Ammonia N.	Amino N.
		gm.	*gm.*	*gm.*	*gm.*
I	1, 2	0.195	0.159	0.006	0.011
II	3	0.13	0.081	0.003	0
	4	0.17	0.109	0.005	0
	5	0.133	0.087	0.003	0
	6	0.126	0.083	0.0	0
	7	0.162	0.067	0.0	0
	8	0.199	0.071	0.003	0
	9	0.36	0.112	0.002	0

Amount of N injected as glycine, *gm*	1.19
" " N recovered " " *gm*	0
Injected glycine excreted unchanged, *per cent*	0
Amount of extra N in urine resulting from injection (total), *gm*	0.6
Injected N recovered in urine, *per cent*	50.4

This is a distinctly subtolerant rate of glycine injection. The urea excretion is still high at the end. Ammonia excretion shows no marked changes.

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FAT EMBOLISM FOLLOWING TRAUMA TO BONES

An Experimental Study of Its Production and Prevention with Particular Reference to the Albee Operation

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THE importance of fat embolism following bone injuries has recently received considerable emphasis. Buerger (1) examining the lungs of one hundred individuals who had received severe contusions or bone fractures found embolic fat lacking in only one case of severe bone injury. In his excellent monograph, Warthin (2) reports that in the eight fatalities following fractures in his pathological service, on which autopsies were performed, "all showed a marked fatty embolism as the cause of death." It is of significance to note that a clinical diagnosis of fat embolism was not made once in these cases. Le Count and Gauss (3), studying 14 cases of fat embolism, found that the symptoms had been recognized clinically only once, while the diagnosis of delirium tremens had been made eight times.

The tragic nature of fatal fat embolism in orthopedic operations has given especial sig-

nificance to these cases. The dangers associated with forced straightening of ankylosed joints were pointed out by de Quervain (4) in 1904 and prophylactic measures were advocated which seemingly attracted but little attention. Von Aberle (5) made similar but more extensive observations in 1907 and outlined means of prophylaxis which are empirical in nature but have had important bearing on orthopedic procedures. The number of well-authenticated fatal cases reported, as found by Heck (6) in 1913 to be within the limits of a score, doubtlessly serves to illustrate the failure to recognize the condition rather than the rarity of its occurrence. Reiner (7) states that four deaths from fat embolism occurred in the Vienna Institute for Orthopedic Surgery alone, within the space of a few years and that three of these deaths occurred in approximately one thousand operations which were performed previous to the adoption of certain prophylactic measures. Ryerson (8) within the past three years has had three deaths, in his own service, which he attributes to fat embolism. Only in the last one of these was the cause of death definitely established. Fat embolism was not suspected at the time, in the two earlier fatalities, and consequently, no examinations were made which would reveal it. One of these cases is of peculiar interest in connection with these experiments. It was an Albee bone transplantation for the correction of a severe paralytic scoliosis. A splint cut from the tibia by means of a motor saw was sewed into a cleft made by splitting

the spinous processes of the lower dorsal and upper lumbar vertebræ. The death which occurred three days later is the first one following this type of operation in which fat embolism has been reported and was responsible, in part, for the present study, undertaken at Dr. Ryerson's request.

EXPERIMENTAL PRODUCTION OF FAT EMBOLISM

The relationship which fat embolism holds to lesions of bone tissue was established by the experiments of Busch in 1866, who produced fat embolism in rabbits by boring holes in the tibias and destroying the marrow by means of a wire introduced through these holes in the cortex of the bones. These injuries to the bone marrow were followed constantly by the finding of fat droplets in the capillaries of the lungs. The animals killed at the end of three to six hours had similar amounts of fat, with the same wide-spread distribution in the lungs, as had the animals which were allowed to live several days.

Busch investigated also the method of transportation of this fat. The marrow was in part removed from the marrow cavities and replaced by an intimate mixture of olive oil and vermilion. Large amounts of the colored fat were found in the lungs as early as 45 minutes after the completion of the operation. In these cases, no traces of pigmented fat were found in the lymph-glands along the vena cava; and the lymph-glands in the pelvis contained only small quantities of pigment.

In other animals, the vena cava was ligated below the entrance of the renal veins and a cannula inserted into its peripheral end, previous to the injection of the colored fat into the marrow cavities. When the oil was purposely injected under high pressure, the blood flowing from the cannula is said to have contained pigmented fat before the injection was completed, while three hours after the injection it was impossible to find pigmented fat even in the vessels nearest to the injured bone. On the basis of these experiments, Busch concluded that the fat is taken up from the marrow cavity largely by the veins and that the maximum of the absorption is reached during the first few hours. A much smaller amount of fat is apparently transported by the lymph.

Riedel (10) confirmed the finding of Busch that injuries to bones are constantly followed by the entrance of fluid fat into the blood stream and, in addition, showed that the intact lymph-vessels are capable of taking up fat which has been injected into the subcutaneous tissues. He never observed the production of fat embolism by this means and explained this by the emulsifying action of the lymph-glands.

While he did obtain pulmonary fat embolism with intraperitoneal oil injections, he attributed this to the supposed direct entrance of some of the lymphatics of the diaphragm into the thoracic duct without passage through lymph-glands. Fritsche (11), investigating this claim recently, could find no authority for such assumption, and states

that Bartels has shown that all of these lymph-vessels pass at least one set of lymph-glands before reaching the thoracic duct. Wiener (12), likewise, obtained abundant pulmonary fat embolism by the injection of fat into the peritoneal cavity of rabbits. The lymphatics of the diaphragm were noticeably filled with fat. Olive oil injected into the pleural cavities of dogs and rabbits gave a similar result. Some of these animals were allowed to live from 13 to 19 days following the injections, but the experiments were repeated with numerous rabbits, and fat was usually found in the capillaries of the lungs in animals which were killed after two days. These experiments led to the conclusion that even the intact lymph-vessels may take up large fat droplets and that the passage into lymph-glands does not prevent the accumulation of fat droplets in the capillaries of the lungs.

The work of Ribbert (13) demonstrated the importance of jarring the bones without fracture in the production of experimental fat embolism. He found that by striking the bones of the legs of rabbits a series of blows with a mallet, a pulmonary fat embolism was produced. According to his view, the jarring of single bones, or of the skeleton as a whole, is the chief factor in bringing about the entrance of fat into the blood stream. The fat, therefore, would not necessarily come from that part of the marrow which is directly injured by the trauma, as this, he thinks, could account for only a small amount of fat. To show the relatively slight signi-

ficance of the tearing of the bone marrow, when the jarring is reduced to a minimum, he produced fractures in the femurs of rabbits by a very gradually increased pressure. In these animals, no fat embolism was noted, or at most, it was slight, although the fractures were sufficiently complete for the ends of the broken bones to be moved against each other.

Ribbert thought this would explain the occurrence of marked fat embolism, in human beings, following perfectly smooth transverse fractures of bones, in which only a thin layer of marrow is disturbed, and also severe cases following very trivial fractures, or fractures of flat bones whose marrow contains but little fat, such as he had observed in fractures of the sternum, of ribs, and vertebræ, and which he had seen often in skull fractures, resulting from falls and other severe traumas.

Using Ribbert's methods, Frischmuth (14) and later Bergemann (15) found traces of pulmonary fat embolism following the jarring of bones, unaccompanied by fracture. The repeated falling of narcotized animals from a table to the floor, or the throwing of anæsthetized animals against the wall, caused the entrance of free fat into the blood stream but, in every case, the amount of fat found in the lung capillaries was small. In this connection, Bergemann noted that the number of fat droplets, in general, seemed to be dependent upon the age of the animal and the amount of body fat which it possessed. In a few animals, in spite of the most careful

searching of numerous preparations, no trace of fat embolism could be found. These preparations were usually taken from five or six different regions of the lungs and the findings in all of these were generally quite uniform; if traces of fat embolism were found in one of the preparations, they were usually found in the majority of the remaining ones. Taking all possible care to avoid jarring and shaking, Bergemann observed small amounts of free fat in the capillaries of the lungs following gradual crushing of the spongiosa of the long bones of dogs and rabbits, and also after fractures of the shafts of these bones. In these experiments, the fat droplets were larger and more numerous than those seen after the jarring of the animals. He concludes that the crushing of bones, accompanied by fracture, is of more significance in the production of fat embolism than is the jarring of bones and that some importance should be attached even to the compression of bones, since the forced straightening of joint contractures has shown experimentally in man that the compression of bones without jarring may give rise to a fatal fat embolism.

Careful experimental work in the production of traumatic fat embolism has been done also by Fritsche (11). Using rabbits and dogs, he produced fat embolism usually by one of two methods: (1) the method used by Busch of boring holes into the marrow cavity near each end of the tibias, with the destruction of the marrow by means of a wire introduced through the holes; and (2) by

Ribbert's method of striking the anterior surface of the tibias 24 to 36 strokes with a mallet, avoiding injury to the soft parts and jarring of the body in general. Only in a few cases were fractures used to liberate the embolic fat. All of the animals were operated upon in deep morphine-ether narcosis, the excitement stage of ether being avoided by large doses of morphine. While in deep narcosis, they were killed by the opening of both pleural cavities, care being taken to avoid bleeding with a consequent change in the amount of blood in the vessels of the lungs. The time of these experiments, from the liberation of the fat to the killing of the animals, was usually three to four hours; a few animals were allowed to live 18 to 24 hours without any noticeable increase in the amount of fat in the lung capillaries. The examinations of the tissues for fat concerned themselves chiefly with the lungs, since no embolic fat was found in several kidneys examined after three hours.

Usually three or four blocks were removed from the lungs of each animal and sections from each of these were studied. The lungs of a number of rabbits and dogs, killed with the same precautions but without previous bone injuries, contained in some cases a small amount of intracapillary fat. Attention is here called to the fact that it is necessary to differentiate between the large embolic fat droplets and the smaller droplets found in the alveolar epithelium and in the bronchial cells. Fritsche remarks that this finding of embolic fat in the lungs of normal

animals emphasizes anew the fact that a slight grade of fat embolism occurs very readily and is without significance. Only in one animal, were there no demonstrable traces of fat embolism in the lungs after each tibia had been struck 24 blows with the mallet. This was an underdeveloped rabbit, six weeks old, whose bone marrow probably contained but little fat. In an attempt to settle the much disputed question of whether fat embolism arises only through the blood vessels or whether, under certain conditions, the lymph stream can also transmit fat from the bone marrow into the circulation, Fritsche performed a further series of experiments on rabbits and dogs. Before the production of the fat embolism by trauma to the bones, the veins from the legs of five rabbits and one dog were ligated, the collateral vessels being occluded as completely as possible. The results obtained are quite remarkable. By merely striking and jarring the extremities, in spite of the ligation of the veins, the fat droplets in the lung capillaries were found to be as abundant as they were in the controls, indicating a transportation of the fat through the lymphatics.

The importance of this route was seemingly confirmed later by the finding of large fat droplets in the lymph from the thoracic duct of animals similarly treated. On the contrary, when the fat embolism was produced by fractures of the bones or by disturbances of the marrow accompanied by hæmorrhages, the results obtained were quite different.

In these animals, the ligation of the veins prevented the collection of fat in the lung capillaries. With injuries of this kind, the fat embolism apparently arises chiefly by way of the veins. Fritsche suggests that the lymphtics may be easily closed as a result of the hæmorrhage. No hæmorrhage was ever observed in the bone marrow following the jarring of the bones in the earlier experiments. Ligation of the common iliac vein alone was not effective in preventing fat from entering the lungs, following injuries accompanied by hæmorrhage; this, however, was observed in only one animal and then 18 hours after the injury; so, in this case, the fat may have been transported, at least in part, through the lymphatics.

On the basis of these experiments, Fritsche believes that, at least so far as short-time experiments are concerned, the lymph-channels have no important part in the origin of pulmonary fat embolism following injuries involving hæmorrhages into the bone marrow; while, in connection with the jarring of the long bones unaccompanied by a tearing of the blood-vessels, the lymphatics are the chief carriers of the fat. In this latter case, the emboli formed in a given time are always less numerous than when the blood-vessels are torn.

PROPHYLAXIS AND TREATMENT

Numerous suggestions have been made in regard to prophylaxis and treatment in fat embolism, but the actual experimental work done in this field is quite fragmentary.

Probably the earliest attempts in the treatment of an experimentally produced fat embolism were those of Czerny (16) in 1875. Since fat fairly easily forms an emulsion with sodium carbonate solution and, as it was known that emulsions injected into animals produced but little effect, Czerny injected fat into the jugular veins of dogs in amounts sufficient to produce dyspnœa and then introduced into the same veins, varying amounts of 2 per cent sodium carbonate solution, hoping that an emulsion would be formed with the fat in the lung capillaries. While he believed the dyspnœa was momentarily lessened, in some cases, there was no evidence of any permanent relief.

The experimental work of Ribbert (13) emphasized the importance of simple jarring of the bones in the production of fat embolism in healthy animals. He maintained that, if with a marrow which is not especially rich in fat, such as that in the bones of rabbits, this result is obtained, it would certainly occur more readily in human beings whose bone marrow is sometimes extremely rich in fat. While Bergemann (15) was unable to convince himself of the fundamental significance of this jarring and shaking, he admitted that it might be a factor. He states that if this were truly of so much importance, the use of the chisel in surgery would have to be dispensed with because of the attendant danger, and remarks that because of this possible danger, Lexer always preferred the saw to the chisel in operations on the spongiosa of bones, especially atrophic ones. Reason-

ing from the results of experiments similar to those of Ribbert, Wilms (17) attributed to the lymphatics a considerable importance in the transportation of fat. If we maintain that the fat is taken up by the veins, the maximum grade of fat embolism should develop in the course of a few hours, which appears to be contrary to clinical observations; in this case, he states, there would be no other means of combating its origin than to incise the region of the injury in order to remove the fat and the blood which has escaped. He suggests that if the lymphatics serve as the chief means of carrying the fat, benefit might be secured by a temporary drainage of the thoracic duct to the exterior of the body.

This method was used by Wilms with a man 20 years of age who had fallen out of a second-story window and who later developed symptoms of fat embolism. From the fistula produced, two liters of lymph were collected during the first 20 hours after the operation. This fluid is said to have contained large droplets of fat in addition to small droplets presumably derived from ingested fatty materials. Recovery ensued and the fistula closed after four days. This observation led to Fritsche's study of the effect produced by draining the thoracic duct in rabbits and dogs, in which traumatic fat embolism was produced.

The work was limited to three rabbits and two dogs. By striking the tibias, as in the experiments previously cited, no fat droplets were found in the lung capillaries of three animals, when the lymph was led out through

cannulas in the thoracic ducts, while fat droplets were demonstrable in the fluid which escaped. However, the results were quite different when holes were bored into the tibias and the bone marrow broken up, as was done with one dog and one rabbit. In spite of an abundant flow of lymph from the thoracic duct, no fat droplets were found in this fluid, but, on examination of the lungs, fat droplets were found in the pulmonary capillaries. These findings are in accord with Fritsche's conception of the different routes for the transportation of the fat in his two types of trauma to bones. In regard to the suggestion made by Wilms, he states that only in cases of shaking and jarring of the skeleton without bone fracture, would the draining of the thoracic duct be of any prophylactic value, and, in such cases, the symptoms would have to be recognized early.

On a clinical basis, von Aberle (5) in 1907 outlined a method of prophylaxis to be followed in orthopedic operations, which has received wide attention. The chief features of this method are briefly expressed as follows: (1) Since fat embolism following orthopedic operations occurs most frequently with paralytic contractures in extremities which have been long in disuse, he advocates increased care in the prevention of the formation of such contractures and of the recurrence of the condition, when once corrected. (2) Operations should be done as early as possible when once deemed necessary, since with increasing age there is increased fat

content of the bone marrow, as well as danger of bone atrophy. (3) Since many of these operations are done in bloodless fields, with the use of the Esmarch constrictor, it is suggested that the fat collected in the veins may be washed suddenly into the circulation unless the constrictor is removed gradually. (4) When contractures must be operated upon, fractures should be carefully avoided and multiple contractures should never be treated all at one time; and, in case of high grade contractures, the correction should not be attempted at a single operation. If bones are accidentally broken, they should be set as carefully as possible.

Reiner (7), in the worst of these cases, has used a bloodless field in both open and closed operations, but before the removal of the Esmarch constrictor, has placed a cannula in the saphenous vein in such a way that it extends down into the femoral, so that on removal of the Esmarch, the blood, containing the fat collected from the tissues, is allowed to flow outside. Relatively large amounts of fat are said to have been obtained in this way. However, these suggestions do not seem to have been very widely adopted. Having occasionally observed convulsions following certain orthopedic operations, Schanz (18) is convinced that these might have been avoided if von Aberle's directions were followed; when they have actually appeared, he has used subcutaneous injections of salt solution, from which procedure he obtained the impression that beneficial effects resulted. This treatment is based

on the assumption that the convulsions are due to the plugging up of capillaries in the brain. By this means, the capillaries can be distended and the fat droplets forced onward.

Some years before the publication of von Aberle's prophylactic measures, de Quervain (4) had emphasized the dangers associated with the compression of the spongiosa of atrophic bones and had given directions by which to avoid the dangers associated therewith. He especially recommended the use of the X-rays in diagnosis of the extent of osteoporosis and, if this were extreme, the use of forced straightening should be avoided and more gentle methods applied as previously advocated by Payr. In a recent review of traumatic fat embolism, Tanton (19) holds that the immobilization of fractures is a further factor in prophylaxis; if lipuria and dyspnœa have developed, the opening up of the fracture, with removal of the blood and fat accumulated there, should be considered. Since the beginning of the experimental work here reported, Buerger (20) has advocated the use of the Esmarch constrictor, especially in fractures accompanied by contusions, in order to hinder the further destruction of the fatty tissue by the blood and to prevent the entrance of fat into the veins; by this means also, the clotting of the blood in the crushed fatty tissues would be hastened. By experiments, to which reference only is made, he states that he has shown that fat which has become free is frequently retained by the coagulum when the blood clots. For this purpose, he says the

constrictor would probably not have to be left on longer than a half hour; to hasten the clotting, gelatin might be injected before the removal of the Esmarch, but he opposes this procedure on the theoretical grounds that thrombosis might also be hastened in the vessels containing the emboli. Venesection of the injured limb, previous to the removal of the Esmarch bandage might be of some value, as suggested by Reiner. Buerger even advises Momberg's constriction at the waist line in the most severe cases, especially with crushing injuries of the pelvis.

EXPERIMENTS

The purpose of these experiments was to devise a method which would produce a relatively constant amount of fat embolism by means of trauma to bones and then, with this method, to test the value of the use of the Esmarch constrictor or tourniquet as a prophylactic measure against the fat embolism so produced, and further, to seek other means of limiting the amount of fat entering the circulation. The Esmarch constrictor has long been used for the production of bloodless fields in various operative procedures, but its use as a means of prophylaxis against fat embolism has only recently been suggested and its value never actually determined. In his directions for the prevention of fat embolism, von Aberle suggested that the Esmarch constrictor be removed gradually so that large amounts of fat would not be washed suddenly into the blood stream, but he was referring here only to

those operations in which bloodless fields were being employed and does not mention its use as a prophylactic measure. Reiner, however, states that, in his worst cases, those involving paralytic contractures with marked atrophy of the bones involved, he used the Esmarch in both open and closed operations and before removing it, drained the fat-containing blood from the femoral vein. Previous to the adoption of this procedure, three fatalities from fat embolism occurred in his service. Some months after the beginning of the experimental work here reported, Buerger (20) published an article in which he strongly advocated the use of the Esmarch constrictor in bone fractures to hasten the clotting of the blood in the injured tissues and thus prevent the escape of the free fat.

Large and at least moderately well-nourished rabbits and dogs were used in our experiments.

Production of anæsthesia. To avoid the excitement stage of ether anæsthesia, a narcotizing dose of chloral hydrate was first given to all animals. For this purpose, a solution of uniform concentration was used throughout; 1 cubic centimeter of the solution was equivalent to 0.1 gram of chloral hydrate. This solution given per rectum to rabbits in the ratio of 0.3 grams of the crystals to 1 kilogram of body weight, gave a deep narcosis, beginning in 5 to 15 minutes and lasting usually about an hour. The subsequent administration of ether was accomplished without much resistance on the part

of the animal. With dogs a stomach tube was used and the chloral hydrate solution was introduced directly into the stomach. This method of anæsthesia while uniformly satisfactory with rabbits, sometimes failed with dogs because of the vomiting which frequently occurred. In such cases, chloroform was used to produce the anæsthesia and ether was substituted later.

Killing of animals and preparation of the tissues. In these experiments the animals were usually allowed to live 5 hours following the operation; a few were killed at the end of 2½ and 3 hours and one was allowed to live 10 hours. Some of the rabbits were killed by placing them in a large bell-jar and then gradually increasing the content of illuminating gas in the enclosed air; the remaining rabbits and all of the dogs were given preliminary doses of chloral hydrate, as in the initial anæsthesia, and this was followed by ether or chloroform. The thoracic and abdominal cavities were opened and the vessels from the lungs, heart and kidneys ligated. These organs were then removed with as little loss of blood as possible, in order that their fat-content should not be altered. These intact organs were placed in 10 per cent formalin, where they were allowed to remain for a week. Frozen sections of approximately uniform thickness (about 40 microns) were prepared and stained for fat with a saturated solution of scarlet R in a mixture of equal volumes of acetone and 70 per cent alcohol, as recommended by Warthin (2).

Estimation of the amount of embolic fat. The microscopic examination of the organs for fat was confined chiefly to the lungs, since numerous preliminary observations had shown that in these short-time experiments, fat droplets are not usually found in the capillaries of the kidneys, myocardium, and brain, as had been noted previously by others. Blocks of tissue were taken from each of the lobes of the lungs and several sections were made from each of these. To estimate roughly the amount of fat in the lung capillaries, the fat droplets in low power microscopic fields (Leitz No. 4 eyepiece and No. 3 objective. Magnification 103) were counted. In general, 20 fields were counted in each section or a minimum of 100 fields in each pair of lungs. Where the fat droplets were few, twice this number of fields was examined. The fat droplets in each section, and also in different experiments, varied greatly in size, so an attempt was made to estimate more accurately the amount of fat by recording the approximate diameter of each fat droplet in each field examined. This method was persistently followed in the later experiments, and it was noted that when the fat droplets were numerous, large droplets were proportionately more abundant; when there were few droplets, they were mostly relatively small. For comparative purposes, where only wide differences are of much significance, it is considered sufficient to report only the number of the fat droplets.

Operative procedures and results. To se-

cure a suitable means for the production of a fairly constant and abundant fat embolism, three different operative procedures were used.

1. A slight modification of the method used by Ribbert and later by Fritsche was first tried. When the tibias of rabbits were struck numerous sharp blows with a percussion hammer, only the slightest amount of fat embolism was produced. A few fat droplets were usually found in the lung capillaries of each section examined, but often only 2 or 3 in the entire section of at least 1 square centimeter. This amount was, therefore, entirely inadequate for attempts at prophylaxis.

2. A considerably greater but still insufficient amount of fat embolism was then produced by a method similar to that used by Busch. A hole was bored in each end of each tibia and the marrow partially broken up by a wire introduced through these holes into the marrow cavity. In sections made from the lungs of two rabbits, the fat droplets found in the capillaries averaged only 1.5 per low power microscopic field.

3. The following method was then adopted: After cutting the sciatic nerves in rabbits in order to prevent pain subsequent to narcosis, the tibias were crushed throughout practically their entire length by means of a small pipe-wrench, each leg afterward being struck approximately 36 light taps with a small wood mallet.

This is the method which was used in the first series of experiments on prophylaxis,

although the amount of fat found in the lung capillaries following such procedure was not as abundant as desired for this purpose. Upon recovery from the anæsthesia, some of the animals were given liberty to move around freely, while in other cases, especial care was taken to keep them quiet, to see if the amount of pulmonary fat embolism could be reduced by this means. Two of the rabbits in the first group were kept suspended in a towel provided with holes through which the legs were passed; the remaining three animals were allowed to move at will. The amount of pulmonary fat embolism is expressed in the average number of fat droplets found per low power microscopic field, as shown in Table I.

TABLE I

Rabbit	Weight Grams	Duration of Experiment Hours	Condition of Animal during Experiment	Average Number of Fat Droplets
1	2700	5	Moved at will	9.1
2	1850	5	Moved at will	14.3
3	1225	5	Moved at will	0.5
4	2250	5	Suspended in towel	8.3
5	1900	5	Suspended in towel	7.9

This table reveals considerable variations in the average number of fat droplets per field in the different animals. Numerous factors, doubtless, are concerned in the differences here noted; but, with the exception of No. 3, the disagreement is not so great as to be fatal for the present purposes, for moderate variations must be expected because of differences in the amount of fat in the bone marrow of different animals, because of the varying amounts of fat freed by the crushing of the bones, because of the much greater

activity of some animals following recovery from the anæsthesia and also, because of irregularities of distribution in the lungs. The small amount of fat in the lungs of No. 3 can be explained, in part at least, by the size and condition of the animal. It was a small, half-grown and poorly-nourished rabbit, whose bone marrow, like the remainder of its body, probably contained only small amounts of fat; perhaps the age factor here is fully as important as the amount of body-fat. However, even including this animal, the general average of the number of droplets per field is 8.0 and the average weight of the five rabbits is 1985 grams. In general, it was noted that, with the same amount of injury, a larger amount of fat embolism is produced in the larger and heavier animals.

No benefit seems to have been gained here by suspending the two rabbits in towels attached to a suitable frame. Whether this procedure had the desired effect of preventing trauma to the injured limbs, may well be questioned. The unnatural position occasioned considerable struggling which, undoubtedly interfered with the occlusion of the vessels by thrombi.

In the next group of experiments, Esmarch constrictors were placed on the legs of rabbits, proximal to the joint between the tibia and femur, previous to the crushing of the tibias. These rubber bandages were about 90 centimeters long and about 2.5 centimeters wide and were applied with sufficient tension to prevent the entrance of blood into the more distal portions of the extremities. They

were removed at varying intervals after the crushing of the tibias and the effects noted, as in Table II.

TABLE II

Rabbit	Weight Grams	Duration of Experiment Hours	Esmarch Constrictor	Condition of Animal during Experiment	Average Number of Fat Droplets
6	2450	5	On full time	Moved at will	0.1
7	2375	5	On 2½ hours	Moved at will	0.5
8	2550	5	On 2½ hours	Moved at will	3.1
9	2550	5	On 2½ hours	Very active	4.1
10	2950	5	On 2 hours	Quiet with chloral	0.5
11	2250	5	On 2 hours	Suspended in towel	2.2
12	2300	5	On 2 hours	Suspended in towel	1.1
13	1875	5	On one hour	Distinctly quiet	1.2
14	1975	5	On one hour	Moderately active	6.9
15	2475	5	On one hour	Moderately active	2.6
16	2420	5	On ½ hour	Moderately active	0.8
17	2100	5	On ½ hour	Distinctly active	6.7
18	2100	5	On ½ hour	Moderately active	15.3

When these results are compared with those in the previous table, it is seen that the amount of fat in these lungs, as judged by the number of fat droplets per field, is distinctly less. The general average per field in this series is 3.5 as compared with 8.0 in the earlier series, while the animals used here were somewhat larger, averaging 2320 grams as compared with 1985 grams. Leaving the rubber bandages on the legs during the entire experimental time almost completely prevented the entrance of fat droplets into the capillaries of the lungs.

This is of significance only in proving that the fat found in the lungs in these experiments has its origin almost solely in the injured limbs. When the Esmarch constrictors were left on 2½ hours and then removed, the amount of fat embolism which developed

during the remaining 2½ hours, even if the animals were allowed to move freely, averaged 2.6 droplets per field, which in this numerical standard is only one third of the number found when no prophylactic measures were employed. During the experiment it was noticed the No. 9 was markedly active, so much so that it attracted especial attention. This may account, at least in part, for the greater fat-content of these lungs.

The next animal in the series was kept quiet with occasional doses of chloral hydrate, after the removal of the constrictors at the end of 2 hours. These combined preventive measures reduced the amount of fat in the lung capillaries almost to a minimum, the average being 0.5 droplet per field. The next two animals of the group, Nos. 11 and 12, were kept suspended in towels after the removal of the constrictors at the end of 2 hours. Moderately low averages were obtained in these cases, but not distinctly less than with the animals which were given their freedom following the removal of the bandages. As previously stated, this towel suspension is not a very effective means of keeping rabbits quiet.

An attempt was then made to see if the same beneficial effects could be obtained by the use of the constrictors for briefer periods, so that its use might be more applicable to the usual orthopedic operations. The rubber bandages were left on the legs of rabbits Nos. 13, 14 and 15 for one hour and then removed. Rabbit No. 13 remained distinct-

ly quiet following the removal of the constrictors, while the other two animals were moderately active. The average obtained with these three animals is 3.6, or slightly less than half the number obtained in those cases in which no prophylactic measures were used.

The Esmarchs were left on the legs of the last three animals of this series only 30 minutes after the crushing of the tibias. In the lungs of No. 16, the number of fat droplets averaged less than one for each microscopic field although the animal did not remain distinctly quiet following recovery from the anæsthesia; the note recorded at the time of the experiment being "only moderately active." The same note was recorded also for No. 18, in the lungs of which there were found many times as much fat. This difference is seemingly not dependent solely upon the activity of the animals, even though they are of equal size.

The general average obtained for the three animals with which the constrictors were used for ½ hour is 7.6, or only slightly less than the average obtained when no preventive measures were used. This result is not surprising when it is recalled that the entrance of fat into the lung capillaries could be almost completely prevented by continued chloral hydrate anæsthesia, and that the animals here used rarely recovered from the anæsthesia during the first half hour after the operation. In these cases, therefore, the Esmarchs are removed before the animal begins to move about, previous to which

time but little fat enters the lung capillaries as indicated by subsequent experiments.

In another series of rabbits, further observations were made on the effect of narcotic doses of chloral hydrate in preventing fat embolism. The experimental time was here varied from the usual five-hour period (Table III).

TABLE III

Rabbit	Weight Grams	Duration of Experiment Hours	Esmarch Constrictor	Condition of Animal	Average Number of Fat Droplets
13	1350	2½	Not used	Moved at will	8.1
14	2450	3	Not used	Moved at will	11.3
15	2650	3	Not used	Quiet with chloral	0.9
16	2900	3	Not used	Quiet with chloral	1.2
17	2550	3	On full time	Quiet with chloral	0.5
18	2450	10	Not used	Moved at will	3.2

The first two rabbits in this series seem to show that the fat droplets are as abundant in the lung capillaries at the end of 2½ and 3 hours as they are at the end of 5 hours. It is necessary to note this fact in order to be sure that the lessened amount of fat found in the lung capillaries when the Esmarch constrictors are used, is not due simply to the briefer period allowed for the collection of the fat in the capillaries. Rabbits Nos. 15 and 16 were kept quiet by occasional doses of chloral hydrate during the entire time of the experiment, no Esmarch constrictor being used with these animals. This proved just as effective as the use of the constrictors for the two-hour period. With the constrictors on during the full time as with No. 17, only a very slight amount of fat embolism would

be expected. The result obtained with No. 18 indicates that the number of fat droplets in the lungs of these experimental animals is no greater, and is probably even less, at the end of 10 hours than it is with the briefer three- or five-hour periods. Sections from the kidneys and myocardium of this animal contained moderate amounts of embolic fat; the average of 10 fields from the capsule to the pelvis of the right kidney was 1.1 droplets per field, while 10 fields counted in the myocardium gave an average of 1.3 droplets. Apparently, with the ten-hour experimental period, a large part of the embolic fat passes through the pulmonary capillaries and is widely scattered through the greater circulation.

A comparison of the results obtained with the 7 rabbits which were allowed to move about freely, or were simply suspended in a towel, indicates that a fairly constant amount of pulmonary fat embolism is produced by crushing the tibias of rabbits and that a rough estimation of this amount, for comparative purposes, can be made by counting the fat droplets in many representative microscopic fields. The general average for these animals is 8.5 droplets per low power microscopic field, or when the small, half-grown rabbit No. 3 is omitted, the general average becomes 9.8. If the 6 rabbits are considered, in which cases the Esmarch constrictors were used for a period of 2 hours or 2½ hours, and then removed, the general average is found to be 1.9 fat droplets per microscopic field as compared with 8.5 when no attempt was made

to prevent the entrance of fat into the blood stream.

The fact that only a small amount of fat was found in the lung capillaries of the two animals which were kept quiet with occasional doses of chloral hydrate, without any other precautions being taken, indicates that in these healthy animals, the trauma subsequent to the crushing of the bones is a larger factor in the production of fat embolism than is the crushing itself. The importance of these postoperative traumatisms is shown also in those animals with which the Esmarch constrictor was used. The rabbit which was most active, following the removal of the constrictors, is the one in which the greatest amount of fat embolism occurred; on the contrary, the one which was kept quiet with chloral hydrate, following the removal of the constrictors, had the least amount of all, except one on whose legs the constrictors were left during the entire experimental time.

FAT EMBOLISM PRODUCED BY THE ALBEE BONE TRANSPLANTATION OPERATION

Because of an occasional and unexplained death following the Albee bone transplantation operation in paralytic scoliosis and tuberculous spondylitis (Pott's disease) and because of a possible relationship between fat embolism and postoperative pneumonias, similar operations on animals were planned to determine the amount of fat embolism produced. Wolcott (21) states that out of the 198 operations of this kind performed by Dr. Albee previous to January 1, 1915, one

child of 6 years died the day following the operation, probably from shock; another patient died of status lymphaticus and still another of pneumonia, one week after the operation. One fatality following this type of operation is reported by Ryerson (4) as in all probability due to fat embolism. This apparently is the only case reported to date, in which fat embolism was even suspected. If these operations when performed on experimental animals, produced even a moderate amount of fat embolism, a standard procedure would then be available for subsequent testing of means of prophylaxis.

Methods and results. For these experiments, 18 rabbits and 7 dogs were used; all of which were allowed to live 5 hours after the completion of the operation. The rabbits were anæsthetized as in the earlier experiments; the dogs were similarly treated, except that 0.35 grams of chloral hydrate, per kilogram of body-weight, was given in solution by stomach instead of by rectum. An incision was made through the skin and underlying soft tissues, over the most superficial portion of the tibia and extending from one end of the bone to the other. By means of a motor saw, such as is now commonly used for this purpose, a splint of bone approximately 5 centimeters long and 0.3 to 0.5 centimeters wide, was removed from each tibia of the rabbits. This could be done with very little jarring or disturbance of the bone marrow. The skin incision was then carefully closed to prevent loss of blood. In the complete operation, these tibial splints

were then sutured into a cleft made by splitting the spinous processes of six vertebræ in the lower dorsal and upper lumbar region. This attempted splitting of the narrow spinous processes was not always successful but the amount of trauma to which they were subjected was equalized as nearly as possible.

To determine the amount of fat embolism produced in rabbits by removal of the tibial splints alone, a narrow strip of bone was removed by means of a motor saw from each of the tibias of 4 rabbits. No attempt was made here to produce any unnecessary injury to the bone marrow. A subsequent examination of the lungs revealed only a very limited amount of fat in the capillaries. The general average of the number of fat droplets per low power microscopic field was 0.35, or one fat droplet for three fields. The average in the different animals ranged from 0.1 to 0.6, but this largest amount can be accounted for, in part, differently, since the animal from which these lungs came fractured a leg soon after the operation. It is seen, therefore, that this slight injury to the bones in the removal of splints from the tibias, produces an appreciable but not a large amount of pulmonary fat embolism in rabbits. Table IV shows the results obtained with the different animals.

TABLE IV

Rabbit	Weight Grams	Operation Performed	Average Number of Fat Droplets per Field
19	2350	Splints from tibias	0.4
20	2150	Splints from tibias	*0.6
21	2500	Splints from tibias	0.3
22	2200	Splints from tibias	0.1

* Leg broken.

To see if any fat could be found in the lung capillaries following injuries to the subcutaneous fatty tissues and the trauma due to the splitting of the spinous processes, the spinal part only, of the Albee operation, was performed on 4 rabbits. The incision over the spinous processes was made sufficiently long to give access to six vertebræ in the lower dorsal and upper lumbar region, and the processes were then split with a chisel. The amount of fat embolism produced, as judged by the number of the fat droplets in the capillaries of the lungs, is distinctly greater than that produced by the removal of the tibial splints. The average for the two animals which survived the operation is 1.6 droplets per field as compared with a general average of 0.35 in the earlier series. Two of the 4 rabbits in this group died just before the completion of the operation. The tissues from these animals were preserved in the usual way and the lungs later examined for their fat-content. Even in the brief time that the animals lived, after the beginning of the operation, a considerable amount of fat embolism had developed, the average for the two being 0.75 fat droplet per field. Nothing was found to account for the death of these animals; but, it might be due to mechanical interference with respiration, since they were placed on their bellies during the splitting of the spinous processes. The individual results are shown in the Table V.

Complete Albee operations, including both the removal of the tibial splints and the splitting of the spinous processes, were then per-

TABLE V

Rabbit	Weight Grams	Operation Performed	Average Number of Fat Droplets	Remarks
23	1900	Spinal part only	0.4	Died during operation
24	2300	Spinal part only	1.7	Killed at end of 5 hours
25	2700	Spinal part only	1.5	Killed at end of 5 hours
26	1900	Spinal part only	1.1	Died during operation

formed on 4 rabbits with results as shown in Table VI.

TABLE VI

Rabbit	Weight Grams	Operation Performed	Average Number of Fat Droplets per Field
27	2500	Complete Albee	1.3
28	2450	Complete Albee	1.7
29	2550	Complete Albee	1.7
30	2200	Complete Albee	1.6

The general average of the number of fat droplets per microscopic field is here again 1.6, or exactly the same number obtained when only the spinal part of the operation was done. The latter, however, was the general average from only two pairs of lungs and the apparent error is not great, since the general average obtained from the tibial part of the operation is only 0.35 per field.

Since the amount of fat embolism produced by the removal of the tibial splints was entirely inadequate for use in connection with preventive measures, an attempt was made to obtain a larger, and yet fairly constant amount, by breaking up the marrow of the tibias, after the removal of the strips of bone. Esmarch constrictors were used with 4 of these rabbits to produce bloodless fields; in two cases the rubber bandages were left on during the entire operation, being removed immediately after the wound had been closed

with sutures; while in the remaining two cases, the constrictors were removed before the wound was sewed up, thus giving some opportunity for the escape of the fat-containing blood. The irregularities in the results obtained in this series of animals baffle all attempts at interpretation. The results are given in Table VII.

TABLE VII

Rabbit	Weight Grams	Operation Performed	Esmarch Constrictor	Average Number of Fat Droplets
31	2050	Splints removed and marrow broken up	Not used	0.8
32	2950		Not used	2.8
33	2850		During entire operation	8.1
34	2350		During entire operation	2.1
35	2650		Removed before sewing up	3.1
36	2500		Removed before sewing up	7.7

Fat embolism produced by the Albee operation when performed on dogs. Because of the small amount of fat embolism produced in rabbits by the unmodified Albee operation, it was decided to perform similar operations on dogs. For this purpose, 7 large, mature, and moderately well-nourished dogs were used. Table VIII shows the operations performed and the results obtained.

TABLE VIII

Dog	Weight Kilograms	Operation Performed	Remarks	Average Number of Fat Droplets
1	15.5	Splints from tibias	Marrow broken up	2.5
2	16.5	Complete Albee	Marrow broken up	1.6
3	17.8	Complete Albee	Marrow not disturbed	0.1
4	17.0	Complete Albee	Splints removed with chisel	0.5
5	17.0	Complete Albee	Splints removed with chisel	0.1
6	22.0	Complete Albee	Splints removed with chisel	0.2
7	14.0	Complete Albee	Splints removed with chisel	0.5

When the bone marrow of the tibias was broken up with a probe as with dogs 1 and 2, a moderate but seemingly inconstant amount of pulmonary fat embolism was produced. With the unmodified Albee operation, the tibial splints being removed by means of a saw, the lungs of Dog 3 contained a minimum of free fat, averaging only one droplet for 10 microscopic fields. It was, therefore, apparent that the Albee operation liberates too little fat from the bone marrow of normal dogs, to be used for the testing of prophylactic measures.

Because of the commonly accepted view that the use of the chisel in certain bone operations increases the danger of fat embolism, complete Albee operations were performed on 4 dogs, the tibial splints being removed by means of a chisel. In these operations a comparatively dull chisel was used, and an effort was made to produce as much jarring of the bone as could reasonably accompany the removal of such splints from the healthy tibias of dogs. The results obtained from a subsequent examination of the lungs of these dogs for intracapillary fat, as shown in the above table, gave a general average of only 0.33 droplet per field, or only one droplet for three microscopic fields. This seems to show quite conclusively that the use of the chisel in the removal of splints from the healthy bones of dogs does not increase very appreciably the amount of fat embolism produced, above that produced when the motor saw is used.

DISCUSSION OF RESULTS

The fairly constant numerical values obtained by counting the fat droplets in the lung capillaries, following the crushing of the tibias of normal mature rabbits, is evidence that this procedure when unrestricted gives rise to comparable amounts of fat embolism.

Furthermore, any marked decrease in this numerical value, as observed in a series of animals, can be interpreted as indicating a smaller amount of pulmonary fat embolism. With this as a standard, it is possible to compare the amounts of fat liberated by different operative measures. The striking of the otherwise uninjured tibias is thus seen to produce a very slight amount of fat embolism as compared with the crushing of these bones. Likewise, the destruction of the bone marrow as completely as possible by means of wires introduced into the marrow cavities through holes at the ends of the bones, or the breaking up of the marrow with a probe, after the removal of splints from the tibias, produces a much smaller amount of fat embolism than does the crushing of the tibias. That the latter causes a larger amount of fat to enter the blood-vessels may be due in part to the greater injury to the fat-containing tissues, but it is probably due largely to the greater interference with the clotting of the blood, or rather the disturbance of the clots by the postoperative activities of the animals; the trauma in these cases is exerted on the injured tissues which are no longer protected by intact bone. In support of this is the

result obtained when the animals are kept quiet with chloral, following the crushing of the tibias; no greater amount of fat was found in the pulmonary capillaries of these than was found in others when the marrow was broken up and the bony framework left intact as a support for the injured marrow.

That Esmarch constrictors when left on the legs of rabbits for 2 hours after the crushing of the tibias, distinctly lessen the amount of fat entering the circulation during the remainder of the experimental period, seems apparent from the data here presented. This preventive action may, indeed, be due, as suggested by Buerger, to the earlier and firmer clotting of the stagnant blood, a part of the free fat being retained in the clot and the torn vessels being occluded by thrombi. The fact that activity on the part of the animal, following the removal of the constrictors, may offset to some extent the advantage gained by their use, shows that even after clotting has occurred, trauma to the injured limbs may still cause the entrance of some fat into the blood stream, and emphasizes the importance of early and complete fixation in those cases in which there is danger of fat embolism. The use of the Esmarch constrictors for periods of 30 minutes or even one hour after the operation, furnishes only a slight or uncertain protection against the subsequent development of pulmonary fat embolism in rabbits which are allowed to move about freely after the removal of these constrictors. Those animals which remain distinctly quiet during the whole

experimental period will usually have relatively few fat droplets in their lung capillaries, but this will usually be true as well when the constrictors have not been employed at all. If coagulation of the blood in the injured area is an important factor in preventing the entrance of fat into the blood stream, as is here indicated, it is suggested to us by Dr. Wells that applications or injections of kephalin or brain lipoids (22) made at the site of operation, might hasten the clotting of the blood as discovered by Howell (23), and thus lessen the time necessary for the use of the constrictors.

CONCLUSIONS

1. Crushing the tibias of mature rabbits produces a moderate and fairly constant amount of fat embolism, as determined by counting the fat droplets in a large number of representative microscopic fields, in stained sections of lung tissue.

2. Esmarch constrictors placed on the legs of rabbits, previous to the crushing of the tibias and removed after two hours, lessen distinctly the amount of fat entering the lungs during the remainder of the experimental period; this effect is much less marked and more uncertain when they are removed at the end of a half hour or even one hour.

3. The amount of fat embolism which develops after the removal of the constrictors is dependent largely upon the activity of the animals.

4. Rabbits kept in chloral hydrate narcosis during the entire experimental period,

following the crushing of the tibias, develop only a small amount of fat embolism although the constrictors are not used.

5. The removal, by means of a motor saw, of splints from the tibias of normal dogs and rabbits produces an appreciable but a very small amount of pulmonary fat embolism.

6. The use of the chisel for the removal of the tibial splints from dogs increases very slightly, if at all, the amount of fat entering the circulation.

7. The spinal part of the Albee bone transplantation operation, on normal rabbits, produces more fat embolism than does the tibial part.

We wish to express our indebtedness to Dr. H. Gideon Wells for his helpful direction of this work and to Dr. E. W. Ryerson for suggesting the problem and for furnishing the motor saw used in these experiments.

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THE PURINE METABOLISM OF THE DALMATIAN COACH HOUND.

By H. GIDEON WELLS.

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(Received for publication, June 10, 1918.)

Recently Benedict[1] reported the observation that the Dalmatian breed of coach hound exhibits a marked peculiarity in its purine metabolism, to the extent that it excretes large amounts of uric acid, even when on a purine-free diet. This peculiarity was exhibited by four Dalmatians examined, but a fifth, "obviously not of very pure breed," did not eliminate much uric acid. That a single species of dog should exhibit this characteristic is remarkable, especially in view of the fact that the only other mammals that excrete uric acid in any considerable amounts are man and the anthropoid apes. The Dalmatian coach hound, however, differs also from man and the anthropoids, in that it excretes allantoin in considerable amounts; *i.e.*, from one-half to two-thirds as much allantoin as uric acid. Hence the Dalmatian is unique in respect to purine metabolism.

This fact is of more than academic interest, for it gives us for the first time a laboratory mammal that excretes considerable amounts of uric acid, and hence serves as a possible medium for experimentation under conditions somewhat resembling those obtaining in the human subject. Benedict has already employed the Dalmatian for such investigations. Since other species of dogs differ so radically from the Dalmatian in respect to purine metabolism, there is also opened a most interesting field of investigation in the inheritance of a metabolic process, as exhibited by the products of mating of Dalmatians with other species.

Having found an apparently pure blooded Dalmatian in one of our laboratories it seemed desirable to determine whether it was

[1] Benedict, S. R., *J. Lab. and Clin. Med.*, 1916–17, ii, 1.

equipped with the same outfit of purine enzymes as other dogs, or not. We first made several observations at different times on the uric acid excretion, and corroborated Benedict's findings completely. Uric acid was determined by direct isolation and weighing of the crystalline product, in order to avoid the possibility of any unknown substance being responsible for the results obtained. The animal was a female, the weight being about 13.5 kilos, and it was given a liberal diet, chiefly meat. On this diet the excretion of uric acid was found to be as follows:

	Urine.	Uric Acid.
	cc.	*gm.*
Apr. 25	573	0.3673
May 10	610	0.5132
" 13	740	0.6320
June 15	351	0.4409
July 5	910	0.5425
Average daily excretion		0.499

This rate of excretion, 0.037 gm. per kilo, corresponds to a daily excretion of 2.59 gm. of uric acid by a 70 kilo man.

Benedict found about 0.360 gm. of uric acid excretion daily by his 10 kilo dog, or 0.036 gm. per kilo, even on a purine-free diet. When thymus was fed the uric acid rose to as high as 0.600 gm. of uric acid per day, but at the same time the allantoin nitrogen rose from 0.050 to 0.210 gm. Evidently this dog was able to destroy a large part of the exogenous purines, but not all. When uric acid was injected subcutaneously it was recovered quantitatively in the urine, yet there was also a marked increase in the allantoin excretion. These findings are difficult to explain on the basis of our present knowledge of purine metabolism, and Benedict is obliged to suggest that, "It seems probable that uric acid and allantoin are interrelated in metabolism in other ways than have been heretofore assumed."[2]

[2] This article was written in June, 1917, but its publication was delayed during the author's absence in foreign service. In October, 1917, C. K. Watanabe published a paper (*J. Urol.*, 1917, i 485) on experimental uranium nephritis in two Dalmatian coach hounds, in which animals he observed excretion of uric acid, but in much smaller amounts than found by Benedict or with my dog. Dr. M. H. Givens at Yale has analyzed the urine of two fasting Dalmatians, obtaining figures more

In studying the purine enzymes of this dog the same methods were used as described in previous papers from this laboratory.[8] The dog was killed with chloroform, and the tissues, ground to a pulp, were allowed to act upon uric acid and purines both aerobically and anaerobically, with the following results.

Experiment 1.—80 gm. of liver pulp were added to 0.240 gm. of uric acid dissolved with a minimum amount of NaOH in boiling water. The mixture was digested with toluene at 38–40°C. for 24 hours, with a constant stream of warm moist air bubbling through it. At the end of this time the proteins were removed by coagulation and filtration. The filtrate contained only a very small amount of purine material, in which not a trace of uric acid could be detected. There was recovered from the scanty purine precipitate about 0.045 gm. of xanthine. *The liver of this dog contains uricase.*

Experiment 2.—80 gm. of liver pulp were digested with 0.229 gm. of xanthine under the same aerobic conditions as described above. After the digestion there could be detected no uric acid in the mixture, but a considerable amount of xanthine, 0.150 gm. being recovered. This experiment indicates that the liver of this dog exhibits less xanthine-oxidase activity than is seen in other species of dogs, for ordinary dog liver tissue would have converted the xanthine into uric acid and destroyed it quantitatively under these conditions. The rather low recovery suggests that possibly there has been some xanthine-oxidase activity, but does not establish it, since there are sources of quantitative error in this work.

closely resembling those of Benedict and myself. He has authorized me to publish these results, which were as follows:

Weight of dog. *kg.*	Volume. *cc.*	Uric Acid. N. *mg.*
16	455	214
	190	208
	240	208
10.5	330	232
	335	233
	350	213

These figures show a daily excretion of 0.640 gm. and 0.678 gm. of uric acid for these dogs, or respectively 0 040 gm. and 0.064 gm. per kilo.

[8] Wells, H. G., *J. Biol. Chem.*, 1909–10, vii, 171. Wells, H. G., and Caldwell, G. T., *ibid.*, 1914, xviii, 157.

The recovery of a small amount of xanthine in Experiment 1, furthermore, implies an almost or quite complete lack of xanthine oxidase.

Experiment 3.—Control to Experiment 1. 80 gm. of liver pulp were added to 0.240 gm. of uric acid, in solution, and the mixture was immediately brought to 100°C., and held at this temperature for 30 minutes. 0.205 gm. of uric acid was recovered, after incubating with an air current as in Experiment 1.

Experiment 4.—62 gm. of kidney pulp were added to 0.240 gm. of uric acid and digested aerobically. 0.150 gm. of uric acid was recovered. Possibly there was a slight uricolytic activity by the kidney, but repetition of this result would be necessary to prove it.

Experiment 5.—31 gm. of spleen pulp were added to 0.229 gm. of xanthine and digested aerobically. No uric acid could be recovered or detected but 0.155 gm. of xanthine was found. Evidently the spleen lacks xanthine oxidase.

Experiment 6.—80 gm. of liver pulp were placed in 400 cc. of water containing 0.120 gm. of guanine and 0.154 gm. of adenine. The mixture was digested under toluene, without aeration, for 7 days at 37°. Analysis yielded no guanine, 0.208 gm. of xanthine, no adenine, and 0.172 gm. of hypoxanthine. Evidently the liver possesses guanase, and converts added guanine into xanthine; also apparently it converts some of its own purines into xanthine. It also contains adenase which converts adenine into hypoxanthine.

From these experiments on a single specimen of Dalmatian coach hound, it seems that this animal had uricase in its liver, but probably not in the kidney. This observation indicates that the constant excretion of large amounts of uric acid does not depend upon the absence of uricase from the tissues of this breed of dog, although possibly there is a quantitative deficiency in uricase activity that is not disclosed by our experiments. Further studies are necessary to determine this, but as yet no more Dalmatians have become available. A surprising result is the apparent absence of xanthine oxidase in both the liver and spleen. It is unfortunate that we were unable to examine all the tissues of this dog to learn where it forms its uric acid. The absence of uricase from the kidney is to be expected in view of the free passage

of uric acid through this organ. The presence of adenase and guanase in the liver indicates that in respect to the possession of these two enzymes the Dalmatian does not differ from other dogs, although we do not know whether these enzymes have the same wider distribution as in other species of dogs.

SUMMARY.

In corroboration of Benedict's observation, a Dalmatian coach hound was found to excrete large quantities of uric acid. The liver of this same dog possessed the power of destroying uric acid *in vitro*, indicating that the presence of uric acid in the urine of the Dalmatian does not depend on the absence of uricase in its tissues. The kidney did not exhibit uricolytic activity. Neither the liver nor spleen converted xanthine into uric acid, but the liver deaminized both guanine and adenine.

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[Contribution from the Otho S. A. Sprague Memorial Institute and the Department of Pathology of the University of Chicago.]

STUDIES ON PROTEINOGENOUS AMINES.

I. THE SYNTHESIS OF β-IMIDAZOLYLETHYLAMINE (HISTAMINE).

By Karl K. Koessler and Milton Th. Hanke.

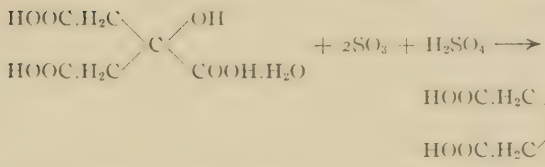
Received August 12, 1918.

The synthesis of β-imidazolylethylamine (β-aminoethylglyoxaline) reported herewith is based on the synthesis of this substance reported by F. L. Pyman.[1] Diaminoacetone dihydrochloride, obtained from citric acid, is heated with sodium sulfocyanide. The thioglyoxaline thus formed, according to Gabriel's[2] general method, is oxidized with nitric acid and through action of nitrous acid formed the hydroxymethylglyoxaline is obtained; over the chloro-compound the nitril is prepared which on reduction yields the amine. While we have followed in the main Pyman's procedure, several additions and improvements have been made which warrant a detailed report of some of the steps involved in the synthesis of this substance which, on account of its remarkable physiological properties, is of great interest to the biochemist.

[1] F. L. Pyman, *J. Chem. Soc.*, 99, 668 (1911).

[2] Gabriel, *Ber.*, 26, 2204 (1893); *Ibid.*, 27, 1037 (1894).

The Preparation of Acetonedicarbonic Acid.[1]



$$HOOC.H_2C\diagdown\ \diagup OH \quad + 2SO_3 + H_2SO_4 \longrightarrow$$
$$HOOC.H_2C\diagup\ \diagdown COOH.H_2O$$

$$HOOC.H_2C\diagdown\ \diagup C:O + 3H_2SO_4 + CO. \quad (1)$$
$$HOOC.H_2C\diagup$$

The method employed by us differs from that of Pechmann in the following details: Finely powdered commercial citric acid containing one molecule of water of crystallization was used instead of the heat-dehydrated substance. To insure the entire absence of water in the reaction mixture, fuming sulfuric acid containing 18.3% of sulfur trioxide was used in the proportion of two pounds of sulfuric to one pound of citric acid. The fuming acid was not diluted with concd. sulfuric acid. The reaction mixture was not agitated. Instead of heating on the boiling water bath, a bath kept at 65–70° was used, the reaction mixture being allowed to lie quietly in the bath. Sulfur dioxide was never evolved at this temperature. The reaction was over about 5 minutes after the cabon monoxide flame went out, and this point was easily recognized by the fact that the solution no longer effervesced. The total time of heating was 55 minutes.

The flask and contents were now cooled to a temperature near 0° and 500 cc. of water added in small portions, the mixture being thoroughly cooled after each addition. After about 200 cc. of water had been added, crystals began to form, and they increased in quantity with each further addition. The resulting semi-solid pasty mass was filtered through art canvas on an 8-inch Büchner funnel and pressed down with a pestle until it was no longer sticky. The product so obtained was over 80% acetone dicarbonic acid. The average yield for 10 experiments was 330 g. of 80% acetone dicarbonic acid from 450 g. of citric acid, which is 86% of the theory. Pechmann obtained 225 g. of equally pure product from 450 g. of citric acid, which is 60% of the theory.

The Preparation of Di-isonitrosoacetone.[2]

$$CO.(CH_2 - COOH)_2 + 2HNO_2{}^3 \longrightarrow$$
$$CO[C(:NOH)COOH]_2 + 2H_2O \longrightarrow CO.(CH:NOH)_2. \quad (2)$$

The method employed by us was a very close duplicate of that used by

[1] Pechmann, *Ann.*, **261**, 155 (1891).

[2] Pechmann, *Ber.*, **19**, 2465 (1886).

[3] The nitrous acid is formed partially by the interaction of sodium nitrite with the acetonedicarbonic acid, but largely by interaction with the sulfuric acid which is present as an impurity in the acetonedicarbonic acid. At first it is the sulfuric acid that furnishes the necessary hydrogen ions. Until it has been completely converted into sodium sulfate, the mixture has a pale yellow color, and carbon dioxide is freely evolved. After the free sulfuric acid has been entirely neutralized, the solution changes color from yellow to bluish brown and the evolution of carbon dioxide almost ceases al-

Pechmann,[1] the only significant difference being in the amount of sodium nitrate used. Operating on 100 g. of crude acetone dicarbonic acid, we found that 60 g. of 93% sodium nitrate was sufficient to complete the reaction. If more nitrate was used the yield was not improved, if less, the product was impure.

The Preparation of Diaminoacetone Chlorostannite.[2]

$$CO(CH{:}NOH)_2 + 5SnCl_2 + 10HCl \longrightarrow [CO(CH_2 \cdot NH_3)_2]SnCl_4 + 2H_2O + 4SnCl_4. \quad (3)$$

Although the principle of the preparation as carried out by us was identical with that described by Kalischer,[3] we found it difficult to obtain uniform results by following the details given in the literature. The large excess of hydrochloric acid present early in the course of the reaction produced a marked decomposition of the di-isonitrosoacetone which gave rise to a brown-colored solution and a decreased yield of the desired substance. The temperature employed by Kalischer was so low that 48 hours were required to complete the reaction. We deemed it best, therefore, to give the process we employed in detail.

400 g. of crystalline stannous chloride was mixed with 110 cc. of 37% hydrochloric acid in a two-liter round-bottomed flask. The mixture became very cold as most of the stannous chloride dissolved. Finely powdered di-isonitrosoacetone was slowly added to this mixture in small portions so that about 15 g. had been added in the course of an hour. The reaction was strongly exothermic. Since the reaction proceeded most rapidly and efficiently at room temperature, it was necessary to cool the mixture somewhat after each addition.[4]

After about 15 g. of di-isonitrosoacetone had been added, the mixture was treated with 110 cc. of 37% hydrochloric acid, which dissolved most of the remaining stannous chloride. The remaining di-isonitrosoacetone obtained from 100 g. of acetonedicarbonic acid, 15–20 g., was then slowly added as described above in the course of another hour.

The final mixture so obtained was of a semisolid consistency, due to the presence of diaminoacetone chlorostannite, which began to precipitate

though the nitrous acid formed is still absorbed. This would seem to indicate that the sodium salt of di-isonitrosoacetonedicarbonic acid does not lose carbon dioxide readily. To complete the reaction it is necessary to add a mineral acid to decompose the above sodium salt. Enough of the mineral acid will have been added when the color of the mixture changes again from bluish brown to yellow.

[1] *Ber.*, **19**, 2465 (1886).

[2] Kalischer, *Ibid.*, **28**, 1519 (1895).

[3] *Ibid.*, **28**, 1519 (1895).

[4] If the mixture became distinctly warm to the touch, decomposition of the di-isonitrosoacetone occurred and poor yields were obtained. If the mixture was too thoroughly cooled, equally poor yields were obtained, probably due to incomplete reduction.

early in the course of the reaction. To insure a complete precipitation of the chlorostannite, 50 cc. of 37% hydrochloric acid was added and the resulting pale brown mixture placed in an ice box overnight. It was then filtered through art canvas on a 6-inch Büchner funnel. The crystals were washed first with conc. hydrochloric acid and then with 95% alcohol to free them from stannous chloride, and adhering mother liquor. Dried at 100° for 5 hours, the perfectly pure, white crystals weighed from 80 87 grams. Since the equivalent of 33 g. of di-isonitrosoacetone was reduced, the above yield is about 83% of the theory.

The Preparation of Diaminoacetone Hydrochloride from the Tin Double Salt.

$$[CO(CH_2 — NH_3)_2]SnCl_4 + H_2S \longrightarrow CO(CH_2 — NH_2,HCl)_2 + SnS\downarrow + 2HCl. \quad (4)$$

Kalischer found it hard to free the solution from tin by using hydrogen sulfide. This was due to the fact that he added hydrochloric acid to the fairly concentrated aqueous *suspension* of the salt. As can be seen from the above equation, hydrochloric acid is liberated in the course of the reaction, and if its concentration exceeded that necessary for dissolving stannous sulfide, precipitation would cease. We found it possible to obtain a product entirely free from tin by *dissolving* 140.4 g. of the tin double salt—0.4 mole—in 3000 cc. of water and saturating the solution with hydrogen sulfide under pressure. The colorless solution obtained after filtering from the precipitated stannous sulfide was freed from water and hydrochloric acid by distillation *in vacuo* at 60 80°. A colorless to pale yellow entirely crystalline solid was obtained, weighing 65 67 g., which is slightly more than the theory—64.4 g.—the excess weight being hydrochloric acid. This product was used directly for the next preparation, which was carried out in the same flask.

The Preparation of 2-Thiol- (4 or 5) -aminomethylglyoxaline Hydrochloride.[1]

$$O:C(CH_2 — NH_2.HCl)_2 + NaSCN \longrightarrow \begin{matrix} HC — NH \\ \| \quad \quad \diagdown \\ C — N \quad \quad C — SH \\ | \\ CH_2 — NH_2.HCl \end{matrix} + NaCl + H_2O. \quad (5)$$

In an attempt to isolate the *free base* of the above substance, Pyman was forced to resort to a rather laborious series of recrystallizations with consequent loss of material. We found that the easily obtainable hydrochloride which was also obtained but not used by Pyman, could be used in place of the free base for the next preparation. Sodium thiocyanate

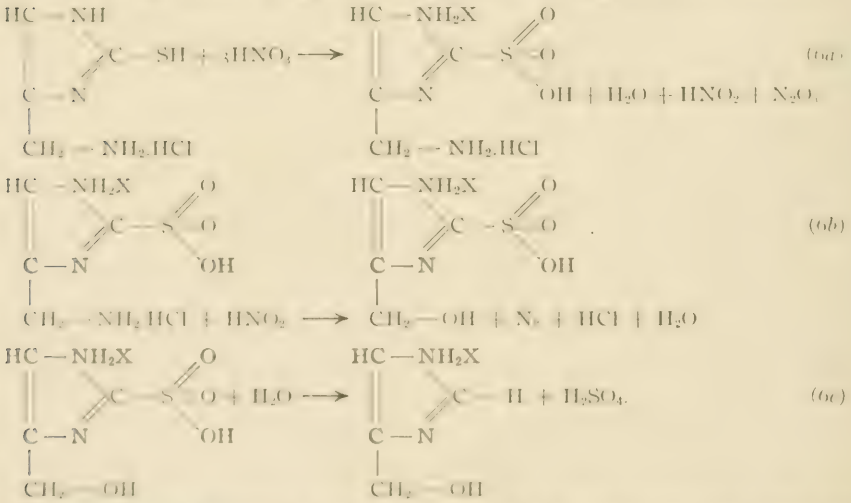
[1] This and the subsequent reactions were first carred out by Pyman and are reported in the *J. Chem. Soc.*, **99**, 668 (1911).

proved to be superior to the potassium salt for reasons that will be given below. The method used was briefly as follows:

The diaminoacetone hydrochloride obtained above, representing 64.4 g. of dry salt, was dissolved in 60 cc. of water and heated on the boiling water bath. 35 g. of dry, chemically pure sodium thiocyanate was added to the solution, one-half immediately, and the rest in the course of 20 minutes. After another 60 minutes of heating the reaction mixture was cooled in an ice bath for two hours and the precipitated 2-thiol-(4 or 5)-aminomethylglyoxaline *hydrochloride* filtered on a Buchner funnel. The crystals were washed free from adhering mother liquor with 50% alcohol in which they are not very soluble. Dried at 100° for two hours the white to pale yellow crystals[1] weighed from 53–56 g., which is 82% of the theory.

The filtrate from the above crystals was freed from water, alcohol, etc., by distillation *in vacuo* at 50°. The resulting solid mass was repeatedly extracted with 95% alcohol to free it from thiocyanic acid, sodium thiocyanate and coloring matter. The crystals (C)[2] which were largely sodium chloride, but which contained some 2-thiol-(4 or 5)-aminomethylglyoxaline hydrochloride, were collected on a filter, dried at 100° and reserved for the next preparation.

The Preparation of (4 or 5)-Hydroxymethylglyoxaline Picrate.



It was found advisable in carrying out the above oxidation to use more water and less nitric acid than specified by Pyman, and to use the *hydrochloride* of 2-thiol-(4 or 5)-aminomethylglyoxaline in place of the free

[1] These crystals were free from sodium chloride. When potassium thiocyanate is used, the product weighs about 5 g. more due to the admixture of potassium chloride.

[2] This is a mark for future reference.

base. By boiling the solution obtained, after the oxidation had been completed, for one hour instead of for 10 minutes, complete hydrolysis of the sulfonic acid derivative was insured. The picrate so obtained was quite pure, and recrystallization was unnecessary.

A concentrated aqueous solution of 16.55 g. of 2-thiol-(4 or 5)-aminomethylglyoxaline *hydrochloride*—0.1 mole—was slowly added to a gently boiling solution of 25 cc. of 70% nitric acid in 400 cc. of water contained in a two-liter flask. The resulting pale yellow solution was then boiled for one hour, no attempt being made to prevent concentration. The final volume was about 20 cc. The well-cooled solution was neutralized with dry sodium carbonate, instead of sodium hydroxide, and poured into a hot solution of 30 g. of 90% picric acid in 700 cc. of water. The mixture was cooled in an ice bath for two hours and the precipitated picrate filtered off on a Büchner funnel and washed with 300 cc. of water. Dried for 24 hours in air the pure picrate melted with decomposition at 207° and weighed 29.5 g., which is 90% of the theory.

It was found possible to conduct an entirely similar oxidation on the solid (C) (see above), by adding the dry solid from two experiments to a single oxidation mixture of 25 cc. of 70% nitric acid and 400 cc. of water. From 20 to 28 g. of pure picrate was always obtained.[1,2]

The Preparation of (4 or 5)-Hydroxymethylglyoxaline Hydrochloride.

Picrates are usually freed from picric acid by treating them with hydrochloric acid, filtering from the precipitated picric acid and extracting the resulting solution with ether. When large quantities of picrates have to be liberated, this process is always accompanied by considerable loss of time and material, and is made disagreeable by the large volumes of ether and acid that it is necessary to employ. By using benzene at its boiling point, we have found it possible to liberate large quantities of picrate without difficulty and without loss of material. This process ought to be applicable to the liberation of any picrate when *the resulting hydrochloride is insoluble in hot benzene.*

100 cc. of 37% hydrochloric acid, 250 cc. of water and 500 cc. of benzene were placed in a two-liter flask and immersed in a water bath kept at 80°. 100 g. of 4-hydroxymethylglyoxaline picrate was rapidly added to this mixture. The contents of the flask were then thoroughly and repeatedly mixed until the solid picrate had passed into solution. This required only a few minutes. The benzene layer, which was nearly sat-

[1] This raises the effective yield of 2-thiol-(4 or 5)-aminomethylglyoxaline hydrochloride to 90% of the theory.

[2] It would be entirely impractical to conduct this oxidation on a similar mixture containing potassium instead of sodium chloride because of the insolubility of potassium picrate. This is perhaps the best reason for using sodium instead of potassium thiocyanate for the preparation of 2-thiol-(4 or 5)-aminomethylglyoxaline hydrochloride.

urated with picric acid, was removed as completely as possible by decantation.[1] The aqueous acid solution was then extracted 5 times in the same flask with hot benzene to remove the picric acid as completely as possible. The last traces of picric acid were removed by heating the pale yellow solution with a few grams of Kahlbaum's highest grade animal charcoal and filtering the cooled solution through a hard, folded, water-soaked filter paper. The residual benzene was left on the filter along with the charcoal and coloring matter. The clear, and usually colorless, solution was freed from water and hydrochloric acid by distillation *in vacuo* at 60-80°. An entirely crystalline pale yellow solid, weighing slightly more than the theory, was left in the flask. The further purification of this solid was accomplished rather simply as follows:

The crystalline mass was loosened from the flask with a steel wire and transferred to a mortar with 100 cc. of acetone. The lumps were rapidly pulverized under acetone, the mixture transferred to a Büchner funnel, and the crystals washed with acetone until they were white. Dried *in vacuo* for 24 hours, the pure white crystals had a melting interval of from 107 to 109° and weighed from 39.8 to 40 g., which is 97% of the theory.

The above rather efficient method for purifying the crude product is applicable only when the pure picrate is liberated. When this is not the case, recrystallization from absolute alcohol has to be resorted to as given by Pyman.

The (4 or 5)-chloromethylglyoxaline hydrochloride and (4 or 5)-cyanomethylglyoxaline were prepared, with no significant modifications, according to the directions given by Pyman. The yields were slightly better than those reported by that author.

The Preparation of Imidazolylethylamine Dichloride (Histamine Dichloride).

$$C_3H_3N_2.CH_2.CN + 4Na + 4HOC_2H_5 \longrightarrow C_3H_3N_2.CH_2.CH_2.NH_2 + 4NaOC_2H_5. \quad (7)$$

The method described by Pyman gave a very poor yield of the substance in the form of picrate. By a radical change in the method we have found it possible to obtain a good yield of very pure histamine in its most desirable form, the hydrochloride.

Five g. of cyanomethylglyoxaline, m. p. 138-40°, was treated with 50 cc. of absolute alcohol in a 500 cc. round-bottomed flask. The flask was suspended from a Liebig condenser by means of a cork stopper, and the alcohol heated to boiling by means of a small flame which impinged against

[1] By distilling the benzene solution of picric acid from a water bath, pure benzene is recovered. The residue of picric acid benzene left in the flask can be converted into picric acid by heating for some hours with water on the boiling water bath. By cooling the solution and adding conc. hydrochloric acid to it, the picric acid can be recovered to the extent of 95%.

an asbestos gauze. A one-half inch air space between the gauze and the bottom of the flask prevented superheating and charring of the sodium ethylate which was precipitated in the course of the reaction. Eight g. of metallic sodium, about two equivalents, was rapidly added in small pieces through the condenser, the time of addition being 5 minutes. After boiling for 45 minutes, 20 cc. of absolute alcohol was added through the condenser to facilitate the solution of the sodium.

After the sodium had dissolved, the mixture was allowed to cool somewhat. 50 cc. of water was then added, and the resulting clear, yellow solution was freed from alcohol, ammonia and some water by distillation *in vacuo* at 50°, until the residual solution had a volume of about 25 cc. The strongly alkaline aqueous solution was then transferred to a graduated separatory funnel with enough water to give a final volume of about 50 cc. This solution was then extracted 5 times with amyl alcohol, using 50 cc. for each extraction.[1] The first 3 extracts were pale yellow in color and contained practically all the histamine.[2,3] The remaining extracts were colorless. The fifth extract was free from histamine.

The strongly alkaline aqueous layer was conserved and used for the preparation of glyoxaline acetic acid (see below).

The combined amyl alcohol extracts were dried over anhydrous sodium carbonate, filtered from the sodium carbonate, using a Büchner funnel, and the clear, pale yellow filtrate extracted 5 times in a separatory funnel with 0.5 *N* hydrochloric acid. 100 cc. of acid was used for each of the first 3 extracts, and 50 cc. each for the remaining two extracts.

The combined aqueous acid extracts were pink in color. Heating for one hour on the water bath changed the color to orange-red. A small quantity of Kahlbaum's highest grade animal charcoal was added to the hot solution. The charcoal absorbed the color entirely. The colorless solution obtained by filtration was freed from water and hydrochloric acid by distillation *in vacuo* at 60°.[4] The pale yellow, slightly gummy, crystalline solid, which still contained a small quantity of sodium chloride, weighed from 7.2 to 7.8 g. It was treated with one cc. of 20% hydrochloric acid, 10 cc. of absolute alcohol, and digested on the boiling water bath. The large lumps were pulverized with a flattened glass rod. The

[1] The two layers must be very completely separated, which by ordinary gravity settling takes fully 20 minutes for each extraction. By using a centrifuge, an even better separation can be effected in a few minutes.

[2] Amyl alcohol extracts histamine quantitatively from a strongly alkaline aqueous solution. The sodium salt of glyoxaline acetic acid remains just as quantitatively in the alkaline aqueous layer.

[3] The presence of histamine was determined by means of Pauly's reaction.

[4] A small quantity of sodium chloride usually separated when the final volume of the solution was about 15 cc. This was removed by filtration after adding 30 cc. of absolute alcohol to the mixture.

coloring matter and gum were entirely soluble in the alcohol, the crystalline solid only slightly soluble. After the resulting mixture had stood for 15 hours in an ice box, it was filtered, the crystals being washed freely with absolute alcohol.

Dried at 100° for two hours, and then finally for 48 hours in a vacuum the pure white crystalline solid had the following properties:

(1) It melted to a clear liquid at 244–246° (corr.).

(2) It was entirely free from ammonium chloride.

(3) It had an intense rose-red imidazole reaction with *p*-diazobenzene sulfonate.

(4) It left a residue on ignition at dull red heat of 3.92%, *i. e.*, 0.5000 g. ignited in a platinum crucible left a residue of 0.0196 g., which proved to be sodium chloride.

(5) 0.1 g. of the solid gave 0.30 g. dipicrate in the form of heavy, flat, truncated pyramids, m. p. 238–240°, from 60 cc. of water. This is the recorded melting point of histamine dipicrate.

(6) A nitrogen determination by the Kjeldahl method indicated that the solid was 94.8% pure histamine dichloride, *i. e.*, 0.6012 g. of solid took 9.28 cc. *N* HCl to neutralize the generated ammonia, as compared to 9.79 cc. demanded for 100% histamine dichloride.

(7) A Van Slyke amino nitrogen determination gave the following results: 0.0619 g. of substance gave 8.15 and 8.20 cc. of nitrogen gas at 24° and 749 mm. If the material was really only 94.8% pure, the actual weight of pure histamine dichloride used was 0.0587 g.

% amino nitrogen. Calc., 7.61%. Found, 7.64 and 7.68%.

(8) A chlorine determination gave the following results: 0.1913 g. of solid took 20.93 cc. of 0.1 *N* $AgNO_3$. If the solid is considered to be 94.8% histamine dichloride and 3.92% sodium chloride, 20.89 cc. 0.1 *N* $AgNO_3$ should have been used.

(9) A large guinea pig died inside of 4 minutes from asphyxia due to bronchial spasm when it was given an intracardial injection of 1 mg. of the substance. A *post mortem* examination showed the lungs to be widely and permanently distended.

(10) An intradermic injection of 0.25 mg. into a 160 lb. man produced the following series of symptoms: There was a marked visceral vasoconstriction within the first 30 seconds, the pulse rising from 80 to 130, with increased facial color. This was followed in the next 30 seconds by a vasodilation accompanied by a rapid drop in the pulse rate, considerable sweating and facial pallor.

A large welt surrounded by a red halo formed around the point of injection. This welt had subsided materially within the first hour but did not disappear entirely until 6 hours later.

A marked spasm of the pylorus gave rise to a feeling of nausea and an expulsion of the perfectly digested stomach contents some 5 hours after the injection.

A very painful headache developed within the first hour and had not entirely subsided 8 hours after the injection.

This series of ten tests proves conclusively that the substance really was histamine dichloride. 4.2 to 4.8 g. of pure histamine dichloride were obtained from 5 grams of cyanomethylglyoxaline, which is from 50–56% of the theory.

A very small second crop of histamine dichloride weighing from 0.15 to 0.20 g., m. p. 210–230°, was obtained from the filtrate from the above solid, which distilled to an oil weighing 2.0 g., by recrystallizing from 3

cc. of absolute alcohol. The purity of this second crop was determined approximately by conversion into the dipicrate. Thus 7.3 g., obtained from a number of experiments, when treated with 21 g. of 90% picric acid dissolved in 2000 cc. of water, gave 15 g. of pure histamine dipicrate, m. p. 241°. The 2000 cc. of solution could contain 1.40 g. of picrate.[1] The maximum total weight would then be 07.2 g. Since 22.1 g. are demanded by theory, the material must have been about 33% pure.

The Separation of Methyl Glyoxaline.

The filtrate from the above two crops of histamine dichloride was freed from alcohol by distillation *in vacuo* at 60°. The combined oil from the reduction of 65 g. of cyanide was dissolved in water, treated with 15 g. of sodium hydroxide and again subjected to distillation *in vacuo*. The semi-solid mass was then extracted repeatedly with ether, about 2500 cc. of ether being used in all. The pale yellow ethereal extract was dried over sodium hydroxide and the ether removed by distillation. 14. g. of pale yellow oil was left in the flask, which was proved to be (4 or 5)-methylglyoxaline by converting it into the picrate. There was thus obtained 36 g. of picrate, m. p. 159-160°. The recorded melting point of methyl glyoxaline picrate is 159–160°.

The Separation of Glyoxaline Acetic Acid.

The strongly alkaline aqueous solution obtained from the reduction of 65 g. of cyanomethylglyoxaline, freed from imidazolylethylamine and methylglyoxaline by extraction with amyl alcohol, was treated with 57% hydrochloric acid until the resulting solution was strongly acid. The solution was then freed from sodium chloride by concentrating *in vacuo*, adding alcohol and filtering. The filtrate from the sodium chloride was freed from water, alcohol and hydrochloric acid by distillation *in vacuo* at 70°. The residue left in the flask was then extracted with a small quantity of hot methyl alcohol, which dissolved the glyoxaline acetic acid hydrochloride readily, but left most of the sodium chloride behind. The brown solid left after the methyl alcohol had been removed by distillation was dissolved in water and the solution decolorized with animal charcoal. The resulting colorless solution was freed from water by distillation *in vacuo* at 60°. The dry, white solid left in the flask weighed 14.25 g. It was triturated in the flask with 20 cc. of hot absolute alcohol, set aside in an ice box to crystallize for 15 hours, and filtered. There was thus obtained 11.1 g. of white needles, m. p. 220-222° (corr.), which were nearly pure glyoxaline acetic acid hydrochloride. One recrystallization from 5 cc. of water and 25 cc. of absolute alcohol gave 7.0 g. of very pure substance, m. p. 226–228° (corr.).

[1] Unpublished data.

THE APPROXIMATE AVERAGE QUANTITIES OF THE PRODUCTS THAT CAN BE OBTAINED FROM TEN POUNDS OF CITRIC ACID.

	Obtained, grams
Citric acid	4530
Acetone dicarbonic acid	3300
Di-isonitroso acetone	1089
Diamino acetone chlorostannite	2640
Diaminoacetone hydrochloride	1200
2-Thiol-4-aminomethylglyoxaline hydrochloride (pure)	950
4-Hydroxymethylglyoxaline picrate	
(1) From the above 950 g. 2-thiol compound	1670
(2) From the solid (C) (see experimental part)	185
4-Hydroxymethylglyoxaline hydrochloride	740
4-Chloromethylglyoxaline hydrochloride	700
4-Cyanomethylglyoxaline	200
Imidazolylethylamine dihydrochloride	165

CHICAGO, ILLINOIS,

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Vol. XL. No. 11. November, 1918.]

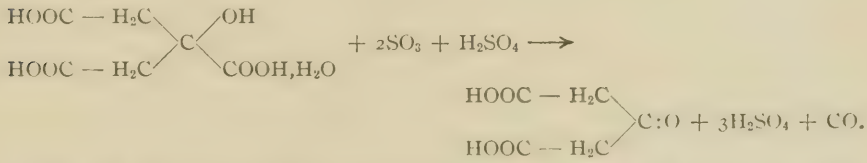
[Contribution from the Otho S. A. Sprague Memorial Institute and the Department of Pathology of the University of Chicago.]

THE ELECTRONIC CONSTITUTIONS OF ACETOACETIC AND CITRIC ACIDS AND SOME OF THEIR DERIVATIVES.

By Milton Th. Hanke and Karl K. Koessler.

Received August 12, 1918.

The first step in the synthesis of imidazolyl ethyl amine[1] is the preparation of acetone dicarbonic acid by treatment of citric acid with fuming sulfuric acid.

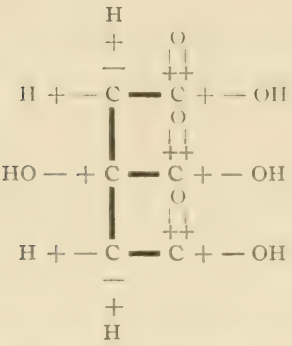


It will be noticed from the above equation that it is the central carboxyl group that is removed as carbon monoxide. This selective action toward the central carboxyl group is hard to explain unless the electrical constitution of citric acid is considered. The following formula shows how much of the electrical constitution of citric acid can be directly foretold by inspection and by a knowledge of its simplest reactions.[2]

It will be noted that the unknown electrical charges are limited entirely to the linkages between the carbon atoms. In attempting to solve the problem of the direction of electrical field in these bonds, the easiest point of attack is the union between the carboxyl groups and the carbon atoms to which they are attached.

[1] See preceding article.

[2] The heavy dashes represent electrical fields of undetermined polarity.



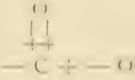
When citric acid is treated with fuming sulfuric acid at 60–70°, all water being excluded (see preceding article), the central carboxyl group escapes as carbon monoxide. The two end carboxyl groups are unaffected. There is no charring of the citric acid nor of the product formed from it, acetone dicarbonic acid. Sulfur dioxide is not formed if the mixture is not superheated. The reason for this selection of the central carboxyl group must be due to a difference in the direction of the valence force holding this group from that holding the two end carboxyl groups.[1]

Carbon monoxide must have one or both of the following electrical structures:

$$C^{\pm}{=}O \text{ (1) or } {}^{\pm}C^{\pm}{=}O \text{ (2).}$$

Formula 1 represents the inactive form of carbon monoxide, inactive because of the entire absence of free valences. Formula 2 represents the active variety of carbon monoxide, which, because of the presence of free valences, can unite with other substances. Although most of the carbon monoxide may be in the inactive form, some of it is certainly in the active form, which is proven by the addition reactions of this substance. When carbon monoxide unites with other substances, it always absorbs one positive and one negative group.[2] The absorption of potassium hydroxide to give potassium formate is a good example of this kind of absorption. This fact proves that the electrical structure of active carbon monoxide must be Formula 2.

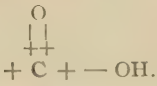
The central carboxyl group in citric acid must therefore have the electrical constitution



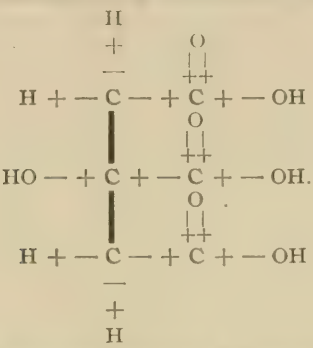
If the end carboxyl groups differ from the central one, they must differ in electrical charges and there is but one possible difference; they must have charges as follows:

[1] Harry Shipley Fry, This Journal, 34, 664 (1913); 36, 256 (1914).

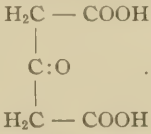
[2] This is true for certain, of course, only when the substance absorbed is neither an oxidizing nor a reducing substance.



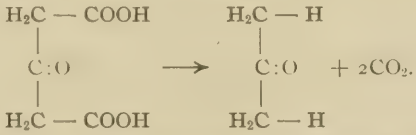
The known electrical constitution of citric acid would then enlarge to



That this is actually the correct formula is proven by a study of acetone dicarbonic acid, which is obtained from citric acid by treatmemt with fuming sulfuric acid. Its structural formula is

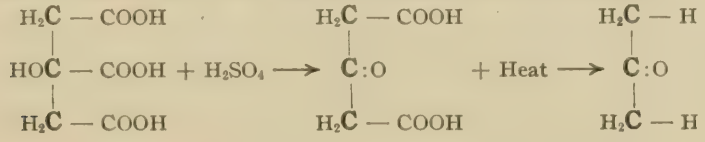


When this acid is boiled in water solution, warmed in mineral acid or caustic alkali solution, or melted, it breaks up into two molecules of carbon dioxide and one of acetone. The same decomposition also occurs when its salts are kept at room temperature.



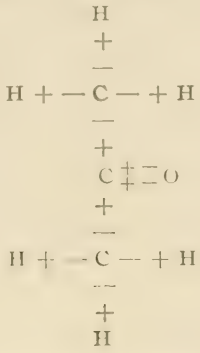
The two carboxyl groups are eliminated as carbon dioxide, not monoxide, which proves that their carbon atoms must have held 4 positive electrical charges since carbon dioxide must have the formula

In the above formula for citric acid, there still remain two bonds whose polarity has not been determined. That the problem of solving hese valences is identical with that of solving the electrical structure of acetone can be readily seen from the following schematic formulas:



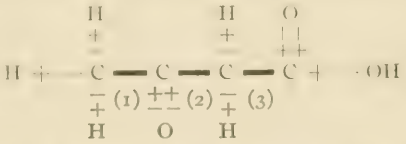
The nucleus of the citric acid molecule is really acetone.

From an inspection of the formula and chemical behavior of acetone, one would be strongly inclined to believe that it was a perfectly symmetrical molecule having the following electrical structure:

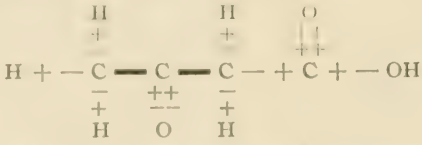


That this is actually the case can be proven by a consideration of the chemistry of acetoacetic acid, acetic acid and keten.

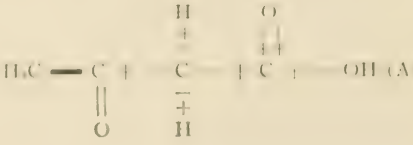
As is well known, acetoacetic acid undergoes two kinds of hydrolysis the one giving rise to acetone and carbon dioxide, the other to two molecules of acetic acid. An inspection of the formula and chemical reactions of acetoacetic acid gives the following electrical formula:



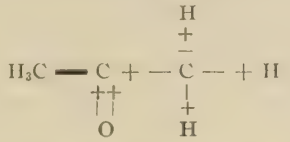
Since carbon dioxide is evolved by hydrolysis and heat, the carboxyl group must contain quadruply positive carbon. Linkage (3) must therefore have the electrical constitution (— +).



Since by another type of hydrolysis linkage (2) is ruptured so that two molecules of acetic acid are formed, the hydrogen atom going to the α carbon atom in the acetoacetic acid, this linkage must have the electrical constitution (+ —).

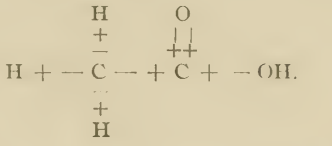


The acetone formed by the hydrolysis of acetoacetic acid must therefore have the following partial electronic structure:

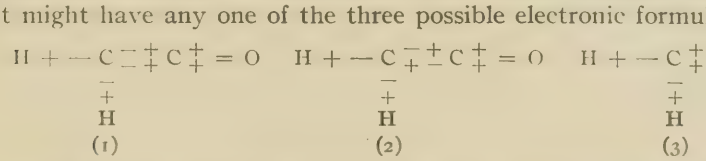


Linkage (1) is apparently still unsolved, but going back to acetoacetic acid, Formula A above, it is clear that the left-hand end of the acetone molecule is electronically acetic acid, because acetoacetic acid gives acetic acid by hydrolysis. If the electronic formula for acetic acid were known, the entire formula for acetone would be self-evident.

By observing the right-hand end of the acetoacetic acid, Formula A above, which by hydrolysis gives acetic acid, the electronic formula for acetic acid would appear to be[1]

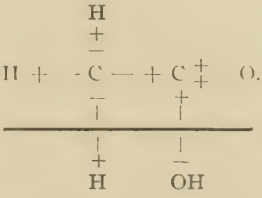


That this is actually the case can be proven by a consideration of the properties of keten.[2] Keten has the structural formula $CH_2 = C = O$. It might have any one of the three possible electronic formulas



(1) (2) (3)

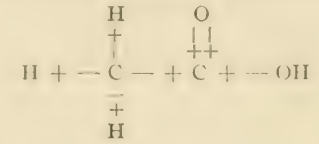
The direction in which water and other substances of undoubted electronic structure are absorbed ought to decide which of these 3 possible formulas is correct. Keten absorbs water readily. If Formula 1 were correct, acetic acid should be the only product.



[1] See also articles by Harry Shipley Fry, *Loc. cit.*

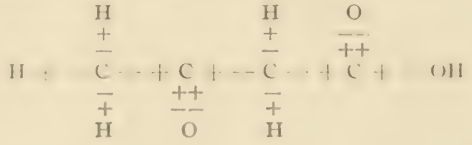
[2] Wilsmore, *Proc. Chem. Soc.*, **23**, 229 (1907); *J. Chem. Soc.* (*London*), **91**, 1030–41 (1907); H. Standinger and H. W. Klever, *Ber.*, **41**, 594–600 (1908); F. Chick and N. T. M. Wilsmore, *Proc. Chem. Soc.*, **24**, 77–78 (1908).

If Formula 2 were correct, a mixture of acetic acid and glycollic aldehyde should be obtained. If Formula 3 were correct, only glycollic aldehyde should be obtained. As a matter of fact,[1] not a trace of glycollic aldehyde is formed, the only product being acetic acid. As further evidence of the correctness of these conclusions, the following might be convincing: With alcohols, keten gives esters of acetic acid; with amines, acetamides are formed. Phenylhydrazine gives the phenylhydrazide of acetic acid; hydrogen chloride gives acetyl chloride and hydrogen sulfide gives the anhydride of thioacetic acid. These entirely one-sided reactions can be explained only on the assumption that the electronic Formula 1 for keten is correct. It follows then as a natural corollary, that acetic acid must have the very symmetrical electronic formula

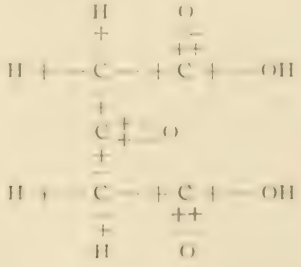


and that the two ends of the acetone molecule are perfectly symmetrical, giving to that substance the electronic formula

The complete formula for acetoacetic acid must then be

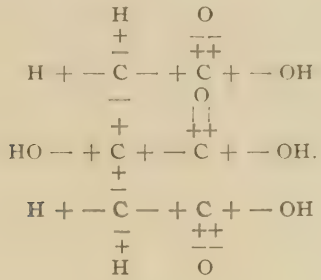


that of acetone dicarbonic acid



and that of citric acid

[1] *Loc. cit.*



CHICAGO, ILLINOIS.

THE EFFECT OF EXPOSURE TO COLD ON ANTIBODY PRODUCTION

ALVIN GEORGE FOORD

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THE EFFECT OF EXPOSURE TO COLD ON ANTIBODY PRODUCTION

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While it is a generally accepted belief that cold is an etiologic factor in the production of disease, especially of the respiratory tract, its effect on antibody production and leukocytic activity has not been studied thoroughly. Numerous experiments have been made to determine the anatomic and pathologic changes produced by cold without the intervention of infectious organisms, and much more experimental work has been done on the effect of cold on the susceptibility to bacterial and protozoon infections, but in most of the latter group of experiments no analyses of the antibody production have been made, mainly because of the fact that most of this work was done before immunology reached its present position. Brief mention of the results obtained by various observers may be made here, the summary of the literature including the results of chilling experiments for other purposes than to determine the influence of cold on the production of immune bodies.

In regard to the anatomic and pathologic changes produced by exposure to cold per se, the work of Walther,[1] in 1862, is perhaps the first. He showed that chilling rabbits until their rectal temperature had decreased 18-20 C. caused a suppression of their secretions, and, if the animals died, hyperemia and edema of the lungs and air passages and a hydrothorax similar to pleurisy with effusion were found. Lassar[2] demonstrated that chilling caused diarrhea in many cases and an albuminuria with hyaline casts 1 to 2 days after the chilling; microscopically the kidneys gave a constant picture of interstitial inflammation. Myer, Lierheim and Siegel[3] caused albuminuria and hematuria by immersing dogs in cold water. Sections of the kidneys showed cloudy swelling, hyaline and necrotic patches and hemorrhage. Preble[4] obtained similar results, using rabbits, and he also found that pleural and pericardial hemorrhages were frequent in animals chilled to death. Aufrecht[5] reports experiments on 3 rabbits in which he thinks he has shown that chilling causes thrombosis and hemorrhages in the lungs.

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1 Virchows Arch., 1862, 25, p. 414.
2 Ibid., 1880, 79, p. 168.
3 Ztschr. f. Exp. Path. u. Ther., 1911, 9, p. 450.
4 Ref. Handbook for Med. Sci., 154.
5 Deut. Arch. f. klin. Med., 1915, 117, p. 602.

No other observers have corroborated the finding of coagulation of the blood in the vessels except Lassar, who found thrombi in the arteries of the viscera of chilled rabbits. Lang[6] did not find any coagulation from freezing localized areas, nor did Hochhaus[7] demonstrate thrombosis of the vessels in the kidney and liver to which he applied — 80° R. for 30 seconds.

Of the work done on establishing the rôle of cold as a factor in disease production and in the progress of disease already begun, Pasteur's experiments in 1878 on anthrax are the first to appear in the literature. These well known experiments were conducted as follows: Hens were inoculated with anthrax and half of them cooled in water, covering one third of their bodies, resulting in a fall of temperature from 42 to 38 C. All the chilled fowls developed the disease while the controls lived. Lock verified the results of Pasteur as did also Wagner,[8] who decreased the temperature by both antipyretics and the cold bath. Sawtschenko[9] obtained the same results with doves, and Dieudonne,[10] Petruchsky,[11] Nuttall,[12] and Metschnikoff[13] have shown that frogs are made susceptible to anthrax if they are warmed up and kept at 37 C.

Lode[14] in an extensive series of experiments found that guinea-pigs chilled in various ways were decidedly more susceptible to experimental infections with the pneumobacillus introduced either subcutaneously or by inhalation, with Staphylococcus aureus, cholera vibrio, B. anthracis and B. tuberculosis, than were controls similarly inoculated but not exposed to cold. Also, the mortality of animals already infected was shown to be increased greatly by exposure to cold. Lipari[15] and Rovighi[16] both found that chilled animals were much more susceptible to pneumococcus infections and the mortality was much higher than in control animals. Dürck[17] found that pneumonia developed without experimental infection in many of his animals which he had chilled in ice water, but never in his controls. Miller and Noble[18] found that chilled rabbits were decidedly more susceptible to B. bovisepticus (snuffles) than controls.

Ernst[19] injected frogs with B. ranicida and found that no infection resulted in the hot summer months, but in the cool spring or fall, or if the frogs were put in the ice-chest, they fell rapidly victims to the infection. Also he showed that if frogs were inoculated at a low temperature and a short time afterward heated up to summer temperature, no disease resulted. Filehne[20] found that after infecting rabbits' ears in the middle with erysipelas, the infection remained latent in the rabbits kept at 0 for 3 days, but severe and extensive erysipelas developed throughout the entire ear when the animal was transferred to room temperature, while in control rabbits the disease was much more moderate. He concludes that the bacteria had progressed to all parts of the ear by means of the lymph channels but that extensive growth was not allowed because of

[6] Abstract in Jour. Am. Med. Assn., 1915, 64.
[7] Virchows Arch., 1898, 154, p. 320.
[8] Centralbl. f. Bacteriol., 1891, 9, p. 322.
[9] Ibid., 1891-1899, pp. 473, 493, and 528.
[10] Arbeiten aus Kaiser. Gesundheitsamt., 1894, 9, p. 492.
[11] Beit. path. Anat., 1888, 3, p. 359.
[12] Ztschr. f. Hyg., 1888, 4, p. 353.
[13] Virchows Arch., 1884, 97, p. 502.
[14] Arch. f. Hyg., 1897, 28, p. 344.
[15] Deut. med. Wchnschr., 1890, 16, p. 44.
[16] Virchow and Hirsch Jahres. gesamt. Med., 1892, 1.
[17] Deut. Arch. f. klin. Med., 1897, 58, p. 368.
[18] Jour. Exper. Med., 1916, 24, p. 223.
[19] Beitr. path. Anat., 1890, 8, p. 213.
[20] Jour. Physiol., 1894-95, 17, p. 315.

anemia from the cold. Ciuca[21] and Vincent[22] demonstrated that tetanus could be produced in white mice inoculated with tetanus spores, by immersing for a considerable time in a freezing mixture. With rabbits, exposure of the entire animal was not sufficient to induce tetanus in animals injected with spores, but exposure of an injected extremity almost invariably resulted in the development of the disease. Vincent explains this phenomenon on the ground that the temperature of the isolated extremity is easily reduced to a temperature at which the phagocytic activity is destroyed, whereas it is impossible to decrease the temperature of the whole body to such a degree.

Ross and Williams[23] demonstrated that rats inoculated with trypanosomes (Evansi, Lewisi and Brucei) resisted the infection decidedly more than controls if they were kept in a cool chamber (20-36 F.). Ross and Thompson[24] showed the same thing for guinea-pigs inoculated with T. gambienzi.

Only two observers have failed to find a decreased resistance to bacterial infections when the host is exposed to cold. Pawlowsky[25] found that intense chilling—17 degrees for 15-30 minutes, failed to cause any decreased resistance to staphylococcus infections in rabbits, although many of his rabbits died from the cold directly. The other observer is Chodounsky,[26] who contests strenuously the belief that cold increases the susceptibility of laboratory animals or man to either artificial or autoinfection, especially referring to diseases of the respiratory tract. He cites his experiments on animals to the effect that pneumonia is not produced any easier in chilled than in control animals; he also presents results of experiments on himself to prove his point. He was 57 years old, subject to catarrh and frequent attacks of bronchitis, but nevertheless exposed himself after a hot bath, without drying, for periods of 20 minutes to an hour in draughts of cold air of 3-12 C. No pneumonia or serious results occurred.

Concerning the effect of cold on the cellular content of the blood, Rovighi, Winternitz,[27] and Schnützen[28] found that in both man and laboratory animals chilling causes a peripheral hyperleukocytosis, especially polymorphonuclear, which lasts for several hours, and Winternitz showed that there is considerable increase of the red cells (and increased hemoglobin index) in the peripheral blood. Rovighi and Secchi[29] showed that excessive cooling resulted in a decrease of the leukocytes, but an increase occurred when the heart action and respiration picked up again.

The conclusion to be drawn from the work on the effect of cold on phagocytosis, is that the leukocytes in chilled animals are less active than those of normal animals; and if leukocytes from normal animals are cooled in vitro their phagocytic activity is decreased. To this effect are the following experiments: Wagner demonstrated that the phagocytic activity of the leukocytes in hens injected with anthrax was reduced to a minimum in hens which had been cooled in a cold bath until the body temperature was reduced 2-3 C. Sanarelli[30] found that the leukocytes of chilled guinea-pigs were less phagocytic to Sp. metchnikovii than those of controls after the animals were chilled, while Lode, studying the

[21] Bull. de l'Acad. de méd., Feb. 17, 1908.
[22] Ibid., 1908.
[23] Ann. Trop. Med., 1910, 4,p. 225.
[24] Ibid., p. 510.
[25] Ztschr. Hyg. u. Infektionskr., 1900, 33, p. 289.
[26] Erkaltung und Erkaltungskrankheiten. Wien, 1907.
[27] Centralbl. f. klin. Med., 1893, 9, p. 1017.
[28] Wien. klin. Wchnschr., 1907, 20, p. 1625.
[29] Münch. med. Wchnschr., 1914, 61, p. 1721.
[30] Ann. de l'Inst. Past., 1893, 7, p. 225.

bacterial content of the leukocytes around the point of injection in the experiments cited, found that the leukocytes of control animals did not contain any more bacteria than the leukocytes in chilled animals. Dean[31] found that the action of opsonin on staphylococci was nearly 10 times as great at 37 as at 6 C., and Rolly and Meltzer[32] found that the phagocytic power of human leukocytes for typical bacilli increased gradually from 6 to 37.5 C., but quite rapidly from 37.5 to 40 C. Trommsdorff[33] found that in guinea-pigs which he injected intraperitoneally with hemolyzed hen corpuscles there was decreased migration of leukocytes into the cavity and that there was also a decreased phagocytosis of the corpuscles in the cooled animals. Ledingham[34] showed that when leukocytes and staphylococci were mixed in the presence of serum, the number of cocci ingested varied as the temperature, from 0 to 40 C., due to the fact that there was less action on the cocci by the opsonins in the serum at low temperatures. After a long sensitization at 18 or 7 C., the action of the leukocytes was quite independent of the temperature within certain limits as 7-18, 37, 40 C.

Practically all of the work on antibody production as influenced by cold has been done since 1906. One of the first series of experiments was that of Graziani,[35] who immunized rabbits with the filtrate from an 8-day broth culture of B. typhosus; three injections of increasing nonlethal doses were given under the skin of the back at intervals of two and three days. Briefly, he found that the agglutinin titer of the serum of animals kept constantly at 2-4 C. was nearly twice that of animals kept at room temperature as controls, the serum being drawn the same number of days after the last injection. In another series of experiments he kept half of a series of rabbits at 32 C. constantly during the experiment, while the other half were kept at 32 C. except for being chilled morning and evening during the period of immunization in water at 20 C. for 30 minutes, after which they were dried and put back at 32 C. The results from this series are similar to those in the first set of experiments, namely, the chilled rabbits showed on the average twice the agglutinin titer of the control animals. Graziani's method of determining the agglutinin titer was a microscopic one, the highest titer being the dilution in which there were no longer distinct clumps of 10-15 bacteria in the midst of free, slightly moving bacilli after 30 minutes.

Fukuhara,[36] in a rather incomplete experiment with 3 rabbits immunized to B. typhosus 31 days previously, found that the agglutinin and bacteriolysin content of the serum was increased in one animal cooled by dipping in cold water, this slight increase persisting for several days. The same result was obtained with a second animal kept at 10 C. for 3 hours, while keeping a third animal at 2 C. for 3 hours caused a higher agglutinin and bacteriolysin titer directly after the chilling, but the animal died on the following day and serum drawn while the animal was dying showed a decreased bacteriolysin titer while the agglutinin titer remained increased.

Trommsdorff, on the contrary, studying the agglutinin and bacteriolysin content of serum 1-8 days after the first injection of B. typhosus in guinea-pigs, found that strong cooling caused a considerable decrease in the agglutinin production. In these experiments the animals were immunized by injections of 1/10 lethal doses of typhoid bacilli, twice in the morning and twice in the afternoon on 2 successive days, and the tests for agglutination were made macro-

[31] Proc. Roy. Soc., 1905, 76, p. 506.
[32] Deut. Arch. f. klin. Med., 1908, 94, p. 335.
[33] Arch. f. Hyg., 1906, 59, p. 186.
[34] Centralbl. f. Bacteriol., I, O., 1908, 42, p. 632.
[35] Ibid., 1906, 42, p. 633.
[36] Arch. f. Hyg., 1908, 65, p. 275.

scopically. Trommsdorff showed also that guinea-pigs immunized to ox red blood cells (2 injections intraperitoneally of 5 c c washed red blood cells) showed a decreased hemolysin production after cooling during the period of immunization. In animals injected after cooling there was a decreased hemolysin production up to the 4th day after cooling. If the first injection was delayed until 5 or 6 days after the cooling there was no effect on the hemolysin production, showing that the effect of the cold had been overcome in that number of days. On a large number of animals Trommsdorff found that there was no constant effect produced on the alexin content of the blood by cooling the animals, as determined by hemolytic experiments. However, if the alexin content was decreased markedly by injection of large amounts of ox red blood cells the regeneration of alexin was very much slower in the cooled animals. He found also that there was a decreased extracellular lysis of partially hemolyzed hen's corpuscles injected into the peritoneal cavity of cooled animals.

Finally, Lissauer[87] found that chilling rabbits, which he had previously immunized with sheep corpuscles, in water at 10 C. to the extent that the body temperature reached 30-35.2 C., caused a marked reduction (about 4 times) in the hemolytic activity of the serum drawn immediately after cooling, compared with the activity of the serum drawn just before chilling. This phenomenon persisted 2-3 hours after the cooling, although tests were not made at later periods.

I. Effect of Chilling on Hemolysin Production for Sheep Corpuscles

Method of Experiment.—In this series 8 rabbits were used, 4 in the chilled group and 4 as controls. Rabbits of similar weight were put opposite one another in the chilled and control groups. All 8 rabbits were immunized with washed sheep corpuscles injected intravenously in doses as follows of 50% suspension at intervals of 2 days: 1 c c, 2 c c, 3 c c, 5 c c. The rabbits in the chilled group were immersed up to their necks in ice water, of which the temperature ranged from 0-8 C., for periods of 7-10 minutes twice a day during the period of immunization. On the days of injection the rabbits were chilled for this period 20 minutes after the injection, this delay being made to allow the immediate shock of the injection to pass off before the chilling was begun, and once again some time during the day after a lapse of 6-8 hours. On the days between the injections the animals were chilled twice, once in the morning and once in the evening. Of the original 8 rabbits, 3 died, one control dying acutely from the third injection, and two chilled rabbits, one dying from snuffles contracted 3 days after chilling began, and the other dying from the effect of cold directly. The first two of these rabbits were replaced by other rabbits treated as outlined. On an average the rectal temperature dropped 8 to 9 C., in one case 12 degrees and in another case 13 degrees, and did not reach normal again for 3 or 4 hours.

Blood was drawn from the ears on the days given in Table 1. In testing the hemolytic power of the serums after inactivation at 58 C. for ½ hour, each tube contained 3 c c, 1 c c being serums diluted with normal salt solution, 1 c c being 2½% suspension of sheep corpuscles and 1 c c being 1/20 guinea-pig serum, diluted with salt solution if necessary to make 1 c c. Before using the complement it was titrated against a known amboceptor so that sufficient but not an excess would be used. All tubes were incubated for 1 hour at 37 C. The figures give the highest dilution at which complete hemolysis occurred (Table 1).

[87] Arch. f. Hyg., 1907, 63, p. 332.

TABLE 1

HEMOLYTIC TITER OF SERUM OF DIFFERENT RABBITS

Days After First Injection	Days After Last Injection	Control Rabbits				Chilled Rabbits			
		1	2	5	7	2	4	6	8
6	...	132	133	133	80	133	133	133	133
9	3	800	800	800	800	800	800	1,333	1,000
12	6	2,000	2,000	4,000	2,000	4,000	2,000	3,000	3,000
16	10	2,000	1,333	3,000	2,000	3,000	1,333	2,000	2,000
20	14	2,000	1,333	2,000	1,000	2,000	1,000	1,000	1,000
23	17*	1,333	1,000	2,000	1,000	1,333	1,000	1,000	1,000
27	21*	400	200	1,333	500	1,000	200	dead	470
35	29*	200	150	1,000	300	500	170	dead	250

On the days marked with the asterisk the chilled rabbits were subjected to chilling 10 minutes at a time, blood being drawn immediately before chilling and immediately after (as in Lissauer's experiments) and the hemolytic titer of the serum determined. The results in Table 2 show that there was practically no difference between the 2 sets of serums.

TABLE 2

HEMOLYTIC TITER DETERMINED BEFORE AND AFTER CHILLING

Rabbit	Day After Last Injection	Titer Before Chilling	Titer After Chilling
2	17	1 : 1,333	1 : 1,333
	21	1 : 1,000	1 : 1,000
	29	500	667
4	17	1,000	1,000
	21	200	170
	29	170	170
6	17	1,000	1,000
	Dead	.....	.....
	Dead	.....	.....
8	17	1,000	1,000
	21	470	470
	29	250	250

From the data in Table 1, which gives the hemolytic titer of the serum from 8 rabbits for a period of 29 days both during and after the period of immunization, it is quite obvious that the chilling as conducted had practically no effect on the hemolysin production in Rabbits 2, 4, 6, and 8. The chilled rabbits ran as parallel to the control animals as possible, there being more individual variation between rabbits in either group than there was difference between the control and chilled groups. Trommsdorff's experience with guinea-pigs is contrary to this result, but his experiments were conducted in a different manner on a different species of animal and consequently there can be little more said in regard to the discrepancy.

Table 2 shows that the hemolysin content of the serum is not modified by chilling a rabbit in water for 10 minutes sufficiently to decrease the rectal temperature 8°–10° C. Lissauer's results, however, are entirely contrary to this result and I cannot explain or account for the discrepancies noted. If it was really the influence of cold which caused the antibody to decrease one-fourth its concentration in the serum on account of chilling in 10° water in Lissauer's work, then it is hard to see why, if Lissauer's report is trustworthy, the rabbits in Table 2, chilled more vigorously than Lissauer's, did not show similar changes. It may be said that in my experiments the serum obtained immediately before and after chilling was titrated at the same time, the same corpuscle suspension, complement and salt solution being used, and the tubes were incubated the same length of time to the minute. Furthermore, it is hard to see how any such influence as cold could cause the removal of three-fourths of the hemolysin from the circulating blood.

II. Effect of Chilling on the Agglutinin Production for Bacillus Typhosus

In this experiment 10 rabbits were used, 5 controls and 5 chilled animals, rabbits of similar weight being placed opposite one another in the 2 groups. The typhoid bacilli to be used in the immunization were grown on plain agar slants for 24 hours, the growth from each tube being washed off in 10 c c normal salt solution, and killed in a water bath at 60 C., for one-half hour. The following doses were given on the first 5 days: 0.25 c c, 0.3 c c, 0.3 c c, 0.35 c c, and 0.5 c c; 0.7 c c was given on the 7th day, 1.0 c c on the 8th, 1.3 c c on the 9th, 1.5 c c on the 10th, and 1.1 c c on the 13th. The animals of the chilled group were chilled as follows: 5 minutes on the 1st day, 6 on the 2nd, 7 on the 3rd, 7 on the 4th, 10 on the 5th, 10 on two occasions on the 6th, 10th and 7th, 12 on two occasions on the 8th, 12 on the 9th, 13 and 10 on the 10th, 12 on the 11th, 12 on the 12th, and 13 on the 13th. The method of chilling was the same as in the hemolytic experiment. The combination of cold and typhoid bacilli proved too much for 3 of the chilled rabbits, No. 4 dying on the 13th day, No. 8 on the 15th, and No. 10 on the 14th day. All the controls retained their weight and were in good health during the experiment except No. 5, which died from an accident on the 19th day.

In testing the agglutinin titer the macroscopic test tube method was used. The organisms to be agglutinated were grown on a large flat surface on plain agar in a large bottle for 24 hours, and then washed off in sufficient salt solution to make the desired turbidity. Before using they were killed by heating at 60 C. for one-half hour in the water bath. In making the tests each tube contained 1 c c of serum diluted with NaCl solution as necessary and 1 c c of typhoid suspension. All serums drawn on the same day were tested with the same typhoid suspension. All tubes were incubated 1 hour at 37 C., and the highest dilution in which agglutination was macroscopically visible was taken as the titer of the serum. Table 3 indicates the results.

TABLE 3

AGGLUTININ TITER ON DIFFERENT DAYS

Days After First Injection	Days After Last Injection	Control Rabbits					Chilled Rabbits				
		1	3	5	7	9	2	4	6	8	10
8	..	3,200	1,200	3,000	3,000	1,600	2,000	2,000	4,000	4,000	2,000
12	..	4,000	3,000	6,000	4,000	5,000	4,000	Dead	6,000	8,000	8,000
15	3	5,000	5,000	6,000	4,000	6,000	8,000	Dead	6,000	8,000	Dead
20	8	4,000	6,000	Dead	5,000	6,000	4,000	Dead	8,000	Dead	Dead
27	15	2,000	2,000	Dead	1,200	2,667	1,200	Dead	2,666	Dead	Dead

On the 15th day after the last injection the 6 rabbits alive at that time were chilled 10 minutes, blood being drawn immediately before chilling, 20 minutes after chilling, and 24 hours after chilling. Titration of the serums obtained gave the results shown in Table 4.

TABLE 4

AGGLUTININ TITER RECORDED AT DIFFERENT INTERVALS AFTER CHILLING

	1	2	3	4	7	9
Before chilling	2,000	1,200	2,000	2,666	1,200	2,666
20 minutes after chilling	2,000	1,600	2,666	4,000	1,600	4,000
24 hours after chilling	2,000	1,600	2,666	4,000	1,600	4,000

From the data in Table 3 it is seen that chilling to the extent described causes a moderately increased agglutinin production for B. typhosus, the average titer in the chilled animals being higher than that of the control series. This was more obvious before the 3 chilled rabbits died, as the comparison of the titers of the first 3 sets of serums shows. Although the agglutinin production was increased the chilled rabbits were more susceptible to the typhoid injections, since 3 of 5 died and the remaining 2 lost more weight and fared much less favorably than the controls. This experiment corroborates Graziani rather than Trommsdorff, whose work on guinea-pigs gave just the opposite conclusions.

The data in Table 4 show that chilling rabbits immunized 15 days previously causes an increased agglutinin content in the serum (except in Rabbit 1), this increase persisting for 24 hours. This work corroborates Fukuhara's results.

ANATOMIC CHANGES PRODUCED IN THE LUNGS BY CHILLING

Aufrecht reported some experiments which seem not to have been repeated, and as a side issue to the chief topic of this paper, the following experiments were made to see whether Aufrecht's observations could be duplicated. Briefly, he reported experiments on 3 animals with no controls which he chilled by immersing in ice water for 1, 3 and 10 minutes, respectively, on each of 3 days. He found that chilling caused coagulation of fibrin in the blood stream in the

part chilled and in the lungs, the coagulation being ascribed by him to the injury to the white corpuscles by the cold. He states also that the coagulation in the lungs gave rise to hemorrhages, especially subpleural, and in the interstitial tissue. Migration of leukocytes was noticed into the adventitia of the vessels and also into the so-called thrombi. From the description the thrombi he observed might well be postmortem clots and artefacts produced by precipitation in the fixing fluid, and the special cell, "neutrophil lymphocyte," answers well the description of a polymorphonuclear leukocyte.

TABLE 5

RESULTS OF EXPERIMENTS TO DETERMINE WHETHER HEMORRHAGES AND BLOOD COAGULATION ARE DUE TO CHILLING

Rabbit	Time of Chilling	Method of Killing	Manner of Death	Macroscopic Changes	Microscopic Changes
1	1 minute on 3 successive days	Blow on neck	Severe spasms, 15 seconds	All showed numerous fresh red hemorrhages especially along margins of lungs, slightly more marked in rabbit No. 3. Hemorrhagic area varied from 1 to 8 mm. in diameter and extended into lung for 1 to 5 mm.	Fresh blood in alveoli in all sections from hemorrhagic areas. No edema No coagulation except one small thrombus 2 mm. in diameter in No. 5. No cellular changes. No other changes
2	3 minutes on 3 successive days	Blow on neck	Severe spasms, 10 seconds		
3	10 minutes on 3 successive days	Blow on neck	Severe spasms, 20 seconds		
4	No chilling	Blow on neck	Severe spasms, 10 seconds		
5	No chilling	Blow on neck	Severe spasms, 15 seconds		
6	No chilling	Injection of ½ gm. KCN intravenous	Extremely severe spasms	Lungs riddled with many fresh red hemorrhages 2-10 mm. in diameter; half of lung tissue hemorrhagic	
7	No chilling	Intravenous injection of chloral (1 gm.)	Quiet death; no spasms or rigor	Several small fresh bright red hemorrhages (2-5 mm.) in upper lobes	
8	10 minutes on 3 successive days	Chloralized completely per rectum; blow on neck	Severe spasms	Several small areas of fresh, bright red hemorrhages in upper lobes; more than in No. 7	
9	10 minutes on 3 successive days	Intravenous chloral injection (1.3)	Quiet death; no spasms or rigor	Only few small areas of fresh hemorrhages; less than No. 7	
10	10 minutes on 3 successive days	Intravenous injection of .66 gm. chloral	Chest opened before death; heart cut open while still beating	One small area of fresh hemorrhages (2 mm.) in each of upper lobes	No changes except small area of fresh hemorrhage noted grossly
11	Same as No. 10	Same as No. 10	Same as No. 10	One small area (2 mm.) only in right upper lobe	No changes except small area of fresh hemorrhage noted grossly
12	Same as No. 10	Same as No. 10	Animal died before chest was opened. No spasms or rigor	Several small areas of fresh hemorrhage as in Nos. 7 and 9	No changes except small area of fresh hemorrhage noted grossly

In my experiments the first 3 rabbits (1, 2, and 3) were treated as Aufrecht's rabbits (Table 5). Rabbits 6 to 9 were narcotized, before chilling, by means of chloral injection per rectum (0.7 gm.). Rabbits 10, 11, 12 were not narcotized before chilling and were treated as follows with the results shown in Table 5.

The first conclusion to be drawn is that the hemorrhages in the lungs were in all cases *fresh* hemorrhages and apparently occurred at the time of death. Furthermore, the hemorrhages occurring in the lungs of rabbits killed by means of a blow on the neck were not due to the chilling, for the controls in all cases showed as much hemorrhage as the chilled animals. Hence, the results indicate that the hemorrhages described by Aufrecht were agonal. In all cases, the amount of hemorrhage in the lung varied with the amount of spasm and rigor as the animal died. The possibility that some of the hemorrhages noticed, especially the more obscure, deeper ones, might have resulted from the struggles in the bath, led to the narcotization of Rabbits 6-9, inclusive, before chilling. However, since the findings in these rabbits are similar to those in the unnarcotized Rabbit 12 killed in a similar manner and to the findings in narcotized rabbits not subjected to cooling (No. 7), and since rabbits not narcotized and which struggled when chilled showed practically no hemorrhages when killed in a different way (Nos. 10 and 11), it is obvious that no hemorrhages resulted from the muscular efforts of the rabbits when being chilled.

Finally, the conclusion is reached that cold did not cause coagulation in the vessels of the lungs, the only thrombus found in the whole series being noticed in a control animal. Numerous structures corresponding to those described by Aufrecht as thrombi were noticed in all the lungs, but they were merely postmortem clots and artefacts produced by fixation.

SUMMARY

Chilling rabbits twice a day during the period of immunization for periods of 7-10 minutes in water at 8° C. did not cause any change in the hemolysin production for sheep corpuscles, and a single chilling 17-29 days after the period of immunization failed to change the hemolysin content of the serum.

Chilling rabbits thoroughly during the period of immunization caused a moderate increase in agglutinin production for B. typhosus. Chilling once 15 days after the period of immunization caused an increase in the agglutinin content of the serum, which increase persisted for 24 hours.

Cold baths in ice water at 0° C. for 1–10 minutes failed to cause hemorrhages or thrombosis in the lungs of rabbits.

THE INFLUENCE OF FORCED DIURESIS IN EXPERIMENTAL POISONING WITH DIPHTHERIA TOXIN *

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This records an effort to apply the principle of forced diuresis or histolavage to the treatment of experimental poisoning with diphtheria toxin. As certain of the bacterial poisons are known to be capable of excretion in considerable amounts by way of the urine, a great acceleration of diuresis might appear to be a more rational procedure in the case of certain infections than in the case of poisoning with heavy metals. Diphtheria toxin was selected because it is sufficiently stable to permit of standardization. The work is reported for the sake of completeness along with that on glaucoma, mercuric chlorid poisoning, etc., since it serves to amplify our knowledge of what may or may not be accomplished by salt and sugar diuresis in general.[1]

TECHNIC

The same general plan was followed as in the mercuric chlorid work.[2] The diphtheria toxin was a stable product 2 years old. The fatal dose was determined intravenously, but not with the same degree of precision as in the case of the mercuric chlorid, owing to the varying resistances found in the different animals. We found that 0.021 c.c. of the toxin per kilogram of body weight killed four out of five dogs in from three and one half to six days. One part of the toxin in

* From the Otho S. A. Sprague Memorial Institute, Laboratory of Clinical Research, Rush Medical College.

1. A fifth detailed report covering phases of work outlined in a preliminary communication (Woodyatt, R. T.; Sansum, W. D., and Wilder, R. M.: Prolonged and Accurately Timed Intravenous Injections of Sugar, THE JOURNAL A. M. A., Dec. 11, 1915, p. 2067).

2. Sansum, W. D.: The Principles of Treatment in Mercuric Chlorid Poisoning, THE JOURNAL A. M. A., March 23, 1918, p. 824.

100 parts of 0.8 per cent. sodium chlorid solution was administered intravenously in each case as a single dose. When diuretic treatment was to follow, the toxin was administered with the first few cubic centimeters of the diuretic solution. Three diuretic solutions were used: (1) an alkaline hypertonic salt solution (sodium chlorid, 2.2 per cent., and sodium

RESULTS OF EXPERIMENTS

No.	Wt. of Dog, Kg.	Intravenous Therapy: Total Dose of Diphtheria Toxin, C.c. per Kg.	Diuretic Solution	C.c. per Kg. Hour	Duration of Injection, Hrs.	Total Amt. Injected	Fluid Used to Preserve Water Balance	Urine, C.c.	Duration of Life, Days
1	11.25	0.021	.....	....	.....	.....	.....	.....	3½
2	8.64	0.021	.....	....	.....	.....	.....	.....	3½
3	12.28	0.021	.....	....	.....	.....	.....	.....	6
4	15.11	0.021	.....	....	.....	.....	.....	.....	6
5	14.28	0.021	.....	....	.....	.....	.....	.....	14
6	11.82	0.030	.....	....	.....	.....	.....	.....	4
7	10.91	0.030	.....	....	.....	.....	.....	.....	5
8*	10.80	0.030	Alkaline salt solution	30	3	1,083	0	963	3
9*	5.11	0.030	Alkaline salt solution	30	4	628	915 c.c. water	827	5
10	20.66	0.021	Glucose, 18%	30	3	1,810	300 c.c. water	2,717	2
11	16.25	0.021	Glucose, 18%, in Ringer's solution	30	3	1,470	478 c.c. water	1,355	Lived
12	15.00	0.021	Glucose, 18%, in Ringer's solution	30	3	1,358	86 c.c. Ringer's	1,056	4
13	17.84	0.021	Glucose, 18%, in Ringer's solution	30	3	1,628	143 c.c. Ringer's	1,410	4
14	24.55	0.021	Glucose, 18%, in Ringer's solution	30	2	1,432	475 c.c. Ringer's	1,670	4
15	11.25	0.021	Glucose, 18%, in Ringer's solution	30	2	646	0	695	4
16	10.57	0.021	Glucose, 18%, in Ringer's solution	30	3	963	0	810	5

* Marked catharsis.

carbonate, 0.371 per cent.; (2) an 18 per cent. glucose solution, and (3) an 18 per cent. glucose solution having the salt content of Ringer's solution (sodium chlorid, 0.7 per cent., potassium chlorid, 0.03 per cent. and calcium chlorid, 0.025 per cent.)

The diuretic solutions were administered as in the experiments described in previous publications. In the first series of four experiments we gave more than

the predetermined fatal dose of diphtheria toxin in the hope of obtaining a decisive therapeutic result. These dogs received 0.030 c.c. of the diphtheria toxin per kilogram of body weight instead of the 0.021 c.c. The two controls (Dogs 6 and 7) lived three and five days, respectively. The first treated dog (Dog 8), weighing 10.80 kg., received 30 c.c. of the alkaline salt solution per kilogram hour for three hours, a total of 1,083 c.c., and passed during that time 963 c.c. of urine. There was also a marked catharsis, which accounts for the urinary output being less than the fluid intake. This dog lived three days. The second treated animal (Dog 9), weighing 5.11 kg., received 30 c.c. of the same alkaline salt solution per kilogram hour for four hours, and also sufficient water from the second machine to prevent dehydration. During the four hours this dog received 1,543 c.c. of fluid, 628 c.c. of the alkaline salt solution and 915 c.c. of water, and passed 827 c.c. of urine and numerous watery stools. This dog lived five days.

We poisoned the remaining seven dogs in this series with the marginal fatal dose of diphtheria toxin, that is, 0.021 c.c. per kilogram. Dog 10, weighing 20 kg., received the 0.021 c.c. per kilogram with the first few cubic centimeters of an 18 per cent. glucose solution, which was given at the rate of 30 c.c. per kilogram per hour for three hours. During the three hours the dog received 1,810 c.c. of the glucose solution and 300 c.c. of water, and passed 2,717 c.c. of urine. This was the greatest diuresis of any in the series. The dog lived only two days, or less time than any other animal in the series. The treatment appeared to have done harm rather than good.

This phenomenon of shortening rather than prolonging life we had noted before in the mercuric chlorid series, and in searching for a possible cause considered the idea that it might be due to the wholesale washing out of urea, chlorids and other essential salts of the body. With the next group of six animals, we poisoned as before, and used an 18 per cent. glucose solution having the salt content of Ringer's solution, preserving the water balance by the addition of extra Ringer's solution when needed. Dog 11 made a complete recovery, but the next five animals (Dogs 12, 13, 14, 15 and 16) died in four, four, four, four and five

days, respectively. We were inclined to believe that the dog which lived had an unusually high resistance for diphtheria toxin, since something similar had been observed in Control Dog 5, which lived fourteen days.

In these experiments, water was passed through dogs at the rate of 30 c.c. per kilogram of body weight per hour for three or four hours. The same procedure, if carried out in the case of a man weighing 70 kg., would imply the intravenous injection of 2,100 c.c. of fluid per hour for a total of 6,300, or 8,400 c.c. in three or four hours, nearly all of this passing promptly out by way of the kidneys and bowel.

CONCLUSIONS

The intravenous injection of an alkaline hypertonic salt solution, an 18 per cent. glucose solution or an 18 per cent. solution of glucose in Ringer's solution failed to prolong the life of dogs poisoned with diphtheria toxin even when treatment was begun at the most favorable time, that is, simultaneously with the administration of the poison. These negative results discourage attempts to apply an identical procedure in clinical cases of diphtheria.

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THE PRINCIPLES OF TREATMENT IN MERCURIC CHLORID POISONING

AN EXPERIMENTAL STUDY *

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The passage through the body of very large quantities of water by sustained intravenous injections of glucose at supertolerant rates has been observed to result in a marked flushing out of urea, chlorids and other substances that are capable of excretion by way of the kidneys. It would seem rational to anticipate that this procedure might perhaps have clinical value in hastening the excretion of certain water soluble poisons. The idea of an actual flushing out of poisons by the production and maintenance of hyperdiuresis is an old one. It has been emphasized by M. H. Fischer,[1] who used particularly saline solutions, and by Turrettini,[2] who reported favorably on the use of intravenous glucose injections in mercuric chlorid poisoning. In connection with other work on the effects of sustained intravenous injections of sugar, salts, etc., at different rates, experiments have been made in this laboratory for the purpose of ascertaining the extent to which one may actually wash poisons of various sorts out of the body by this method.[3]

For experimental study, mercuric chlorid was selected as representative of the heavy metal group of poisons, and diphtheria toxin as representative of a certain type of bacterial poison. A report on the experiments with diphtheria toxin follows.[4]

* From the Otho S. A. Sprague Memorial Institute, Laboratory of Clinical Research, Rush Medical College.

1. Fischer, M. H.: Edema and Nephritis, New York, John Wiley & Sons, 1915.

2. Turrettini: Rev. méd. de la Suisse romande, 1915, **35**, 204.

3. A fourth detailed report covering phases of work outlined in a preliminary communication (Woodyatt, R. T.; Sansum, W. D., and Wilder, R. M.: Prolonged and Accurately Timed Intravenous Injections of Sugar, THE JOURNAL A. M. A., Dec. 11, 1915, p. 2067).

4. Sansum, W. D.: The Influence of Forced Diuresis in Experimental Poisoning with Diphtheria Toxin, THE JOURNAL A. M. A., to be published.

In this series of experiments with mercuric chlorid poisoning, we have studied especially the influence of diuresis; and although no beneficial results were obtained, the study is reported with the belief that it is of clinical interest in showing clearly what can and what cannot be accomplished in the clinical handling of cases of mercuric chlorid poisoning, and to emphasize the uselessness of all known forms of eliminative treatment in cases in which the relatively small but uniformly fatal dose of mercuric chlorid has been once absorbed. The study establishes the minimum uniformly fatal dose of mercuric chlorid with greater sharpness than has been done before. It also brings out the probable reason for the frequent recovery of patients both treated and untreated who have taken quantities of mercuric chlorid which far exceed the minimum lethal dose. It may be stated in the beginning that this study leads us to the belief that efforts to secure the elimination of this poison from the body, once it has been absorbed, are of very doubtful utility, and that the essential indication in the treatment of mercuric chlorid poisoning is to prevent the absorption of a fatal dose.

The glucose solutions, varying in strength from 18 to 54 per cent., were made by dissolving a chemically pure product prepared in this laboratory in freshly distilled water. In some of these experiments sufficient sodium chlorid, potassium chlorid and calcium chlorid were added to the glucose solutions to give them the salt content of Ringer's solution. Hypertonic salt solutions (Fischer's) also were used. The injections were made with a volumetric pump. Two pumps were used in some cases, the second to supply the fluid necessary to preserve the water balance. In these instances the discharge tubes were anastomosed to form a common delivery tube.

The mercuric solutions were 0.1 per cent. (1 mg. per cubic centimeter), dissolved in 0.8 per cent. sodium chlorid.

THE MINIMUM UNIFORMLY LETHAL DOSE OF MERCURIC CHLORID

When this poison is given by mouth, the fatal dose is found to be extremely variable and comparatively large, owing to many factors and especially those

preventing uniformity of absorption (emesis, purgation, the character and amount of food contained in the alimentary tract, etc.). When given by this route, the mercuric chlorid, moreover, passes at the initial absorption rate to the liver, an organ that may differ greatly in its glycogen content and its power to retard the passage of poisons into the blood of the vena cava under varying physiologic conditions. Fantus,[5] working with rabbits, found that in unstarved animals the surely fatal dose by mouth was as high as 40 mg. per kilogram. When given subcutaneously, variations in the absorption time also appear to occur, owing in part to the fact that the tissues at the injection site become necrotic and slough. One of our dogs was given subcutaneously 5 mg. and another 10 mg. of mercuric chlorid per kilogram. Both animals lived, and in both the sites of injection became necrotic and sloughed. By this method, at any rate, we did not succeed in establishing a minimum uniformly lethal dose with sufficient sharpness for a satisfactory control.

To eliminate these uncertainties of absorption, etc., it was decided to determine the least uniformly fatal dose by intravenous injection, and in this way we found a small and very uniform value. Meyer and Gottlieb[6] state that 100 mg. is the minimum dose that has proved capable of causing death in human adults when taken by mouth (1.4 mg. per kilogram on a 70 kg. basis). American textbooks give a higher figure, namely, from 3 to 5 grains, which, for a body weight of 70 kg., would be equivalent to from 2.7 to 4.6 per kilogram of body weight.

The least uniformly fatal dose was determined by us in a series of nine dogs. Each of the first three received intravenously 3 mg. of mercuric chlorid per kilogram as 3 c.c. per kilogram of a 1 : 1,000 solution. The injections administered by means of the volumetric pump were always made to consume exactly one-half hour except when otherwise noted. Two of these animals lived, and the third died in seven days. Each of the second three then received 4 mg. per kilogram as before. All of these animals

5. Fantus, Bernard: Jour. Lab. and Clin. Med., 1916, **1**, 3; 1917, **2**, 3.

6. Meyer, H. H., and Gottlieb, R.: Die experimentelle Pharmakologie, Ed. 2, Berlin and Vienna, 1911, p. 463.

died, the duration of life being four, four and eight days, respectively. None of them developed anuria. The third three dogs each received 5 mg. per kilogram as before. All of these animals died after two, four and six days, respectively. All of them developed anuria. These fatal doses agree approximately with the accepted minimum lethal doses for adult human beings, and in such human cases the inference would be that the patients absorbed all of the dose swallowed. We therefore chose 4 mg. per kilogram as the minimum uniformly fatal dose; and when it was desired to produce anuria, 5 mg. per kilogram were given.

EXPERIMENTS

Our first attempt was to determine whether it was possible to establish a flow of urine in an animal made anuric by the smallest dose of mercuric chlorid capable of producing this phenomenon. A dog was given intravenously 5 mg. of mercuric chlorid per kilogram in the form of a 1:1,000 solution. Twenty-four hours later the dog had passed no urine, but 20 c.c. of thick albuminous urine were obtained from the bladder by catheter. To determine whether the animal was anuric, 200 c.c. of water were given by stomach tube, and four hours later 5 drops of urine were obtained from the bladder. Then 300 c.c. of 0.8 sodium chlorid were given, and two hours later, 3 or 4 drops of fluid were obtained from the bladder. This animal was considered, therefore, to present an anuria of true renal origin. Such a precaution as the foregoing was deemed necessary because in dogs there is often such a suppression of urine associated with the violent catharsis produced by the mercuric chlorid, however given, that the animal may become anuric not from the impaired kidney function but because the available water of the body is deficient.

A 5 4 per cent. glucose solution was then given intravenously at the uniform rate of 5.4 gm. of glucose per kilogram per hour for three hours, but only 10 c.c. of albuminous fluid containing a trace of glucose were obtained from the bladder. A normal animal weighing 10 kg. and receiving the same treatment will, as a rule, pass about 1,000 c.c. of urine. The dog continued to be anuric, and died on the seventh day.

The next experiment was undertaken to ascertain whether any protection could be afforded an animal if treated by copious diuresis immediately following the smallest uniformly fatal dose, that is, 4 mg. of mercuric chlorid per kilogram of body weight, the animal weighing 14.77 kg. This dose of 59 c.c. of a 1 : 1,000 solution was given intravenously at a uniform rate over a period of one hour, and immediately followed by the injection of 19.49 per cent. glucose solution at the rate of 5.4 gm. per kilogram per hour for three hours. During this time, the 14.77 kg. dog received 3,101 c.c. of fluid (1,251 c.c. of the glucose solution with one machine, and 1,850 c.c. of water with the second machine), and passed 2,805 c.c. of urine.[7] The dog continued to excrete urine, but died at the end of the third day. (The diuretic treatment itself does not seriously affect normal dogs).

The same experiment was repeated, with the difference that sufficient sodium chlorid, potassium chlorid and calcium chlorid were added to the glucose solution to give it the salt content of Ringer's solution, and Ringer's solution was used instead of water to preserve the water balance. During the three hours, the 14.77 kg. dog received 2,333 c.c. of fluid (1,353 c.c. of 18 per cent. glucose solution and 980 c.c. of Ringer's solution), and passed 2,125 c.c. of urine. The dog continued to excrete urine, but died in less than forty-eight hours in spite of the active kidney function.

The same experiment was repeated, except that Fischer's solution, as recommended by Weiss,[8] was used immediately following the intravenous poisoning. An 18.4 kg. dog received 30 c.c. of this solution per kilogram of body weight per hour for two hours. During this time it received 1,100 c.c., and the fluid output amounted to 1,000 c.c. of urine and 400 c.c. of liquid feces. (Fischer's solution administered intravenously in the foregoing amounts acts as a cathar-

7. This diuresis in the case of a dog weighing 14.77 kg. corresponds to 1,900 c.c. per 10 kg. of body weight. It is stated in the first paragraph but one preceding that a normal 10 kg. dog receiving the same intravenous and glucose water treatment will, as a rule, pass only about 1,000 c.c. of urine. This dog passed nearly twice as much. The extra diuresis cannot be attributed to the extra water given with the mercuric chlorid, since this was only 40 c.c. per 10 kg. It is due rather to the diuretic effect of the mercury salt itself. The mercuric chlorid, unless given in too large doses, acts as a powerful diuretic.

8. Weiss, H. B.: A Method of Treatment of Mercuric Chlorid Poisoning, The Journal A. M. A., June 2, 1917, p. 1618.

tic.) The dog continued to excrete urine, but died at the end of forty hours.

As these experiments proved entirely negative, so far as beneficial results were concerned, it was deemed futile to continue along this line. Within the past few years, however, a combination of sodium phosphite and sodium acetate has received some attention as a therapeutic agent. Carter[9] reported the successful treatment of many cases of mercuric chlorid poisoning by the oral administration of these salts, and explained their apparently beneficial results on the theory that the sodium phosphite reduced the mercuric chlorid in the tissues to the much less toxic mercurous chlorid (calomel), a reaction that is known to take place rapidly in vitro. Carter's reports[10] are very inadequate, consisting for the most part of mere statements of the number of cases successfully and unsuccessfully treated. But Fantus,[5] working with a large number of rabbits, found these salts of distinct therapeutic value when administered by mouth.

The intravenous injection of sodium phosphite would be the ideal way of bringing this salt into general contact with mercuric chlorid poisoned tissues. A series of experiments was therefore undertaken to determine whether any protection could be afforded in this manner. Carter recommended the use of these salts in the proportion of 10 gm. of the sodium phosphite to 6.6 gm. of the sodium acetate and held that, if the amount of mercuric chlorid taken is known, from five to ten times that amount of the sodium phosphite should be given.

Sodium acetate is a nontoxic substance in everyday use. Sodium phosphite was found by experiment to be nontoxic when administered intravenously in large doses. One dog received intravenously 57 and a second 200 mg. per kilogram of this salt with no apparent toxic symptoms.

One experiment was performed to test the detoxicating power of sodium phosphite in vitro. One gm. of sodium phosphite and 6.6 gm. of sodium acetate were added to 100 c.c. of a 1:1,000 solution of mercuric chlorid. The proportion of sodium phosphite to mercuric chlorid was thus 10 parts by weight of the

9. Carter, T. A.: Chicago Med. Recorder, 1914, **36**, 444.
10. Carter, T. A.: Critic and Guide, 1915, p. 266.

former to 1 of the latter. A white precipitate of calomel soon formed. Of the filtrate, 10 c.c. per kilogram of body weight were given to a dog intravenously with no apparent toxic effects.

Two experiments were performed to determine whether the phosphite solution, when given intravenously immediately following the injection of mercuric chlorid, afforded any protection. The first dog, weighing 14.56 kg., received 58.24 c.c. (4 mg. per kilogram of a 1:1,000 mercuric chlorid solution) over a period of fifteen minutes, which injection was followed immediately by 116.48 c.c. of a solution containing 1 per cent. sodium phosphite and 0.66 per cent. sodium acetate over a period of thirty minutes. Thus twenty times as much sodium phosphite as mercuric chlorid was given. The urine, which amounted to 1,785 c.c. in the first twenty-four hours, contained sodium phosphite. The dog continued to excrete urine, but died in five days with typical lesions. In this case the minimum uniformly lethal dose was given in fifteen minutes instead of the standard thirty minutes, in order not to delay the beginning of the treatment. Our experience would not indicate that variations in the time of injection between fifteen minutes and one hour affect the results.

The second dog, weighing 12.16 kg., received the same dose of mercuric chlorid per kilogram, followed by enough of the phosphite-acetate solution described above to introduce 35 parts by weight of the sodium phosphite to 1 of mercuric chlorid given. This was injected slowly over a period of three hours. The dog continued to excrete urine, but died at the end of four days.

Hall[11] has suggested the use of potassium iodid and quinin hydrochlorid as an antidote for mercuric chlorid poisoning, giving the formula in the accompanying tabulation:

HALL'S ANTIDOTE FOR MERCURIC CHLORID POISONING

	Gm.
Potassium iodid	3.00
Quinin hydrochlorid	1.60
Hydrochloric acid, dilute (10 per cent.)	2.00
Distilled water ..... to	100.00

11. Hall, W. A.: Jour. Am. Pharm. Assn., 1915, **4**, 183.

Such a solution will remove all of the mercury from an equal volume of a 1 per cent. mercuric chlorid solution. Barbour[12] gave intravenously to rabbits a solution similar to the foregoing in sufficient quantity to precipitate all of the mercury from one-half to one hour after having given intravenously from 2.5 to 5 mg. of mercuric chlorid per kilogram; but the animals died sooner than the controls, so that it would appear from Barbour's work that Hall's solution is no more effective in counteracting the effects of mercuric chlorid that has entered the general circulation than those which we have tested.

Another series of animal experiments was undertaken to determine whether the best known forms of alimentary medication afforded any protection following the intravenous poisoning with mercuric chlorid.

The first dog, weighing 8.64 kg., received 4 mg. of mercuric chlorid per kilogram of body weight as before. Carter's[10] antidote was then given my mouth in the proportions and amounts recommended by him, that is, 10 parts of sodium phosphite to 6.6 parts of sodium acetate, and 10 times as much sodium phosphite as mercuric chlorid given. Three such doses were given per day. This dog continued to excrete urine, but died in four days. A second dog, weighing 19.55 kg., was treated in the same way with similar results, continuing to pass urine and living four days. Toward the end of these experiments, both animals vomited parts of the phosphite solution, and to eliminate this possible source of error two rabbits were poisoned and given the same treatment. The first lived seven, and the second, six days. Neither became anuric.

Two dogs poisoned as before were given by mouth the sodium hypophosphite-hydrogen peroxid mixture recommended by Fantus.[5] Three doses were given per day, each dose consisting of 40 mg. of sodium hypophosphite and 2 c.c. hydrogen peroxid per kilogram of body weight. The first dog lived five days, and the second, six days. Both continued to excrete urine.

12. Barbour, H. G.: Mercuric Chlorid Poisoning in Animals Treated Unsuccessfully by Parenteral Administration of Hall's New Antidote, The Journal A. M. A., Feb. [illegible], 1914, p. 736.

Two dogs were poisoned as before and given the Lambert and Patterson[13] treatment, with the sweating necessarily omitted. The reader is referred to their article for the details of this treatment. The essential points are repeated gastric and colonic lavage, diuretic potassium bitartrate sugar solutions by mouth, a continuous rectal drip of 0.8 sodium acetate solution, daily sweats, and albumin in the form of milk. One dog lived three, and the other, two days.

SUMMARY

The minimum uniformly lethal intravenous dose of mercuric chlorid in dogs was found to be 4 mg. per kilogram of body weight (1:1,000 solution injected uniformly during from fifteen to sixty minutes). It would appear that this dose has not heretofore been sharply defined; but it corresponds approximately to the smallest doses of mercuric chlorid which, according to standard textbooks, have been known to cause death in man. The dose of 4 mg. per kilogram uniformly failed to cause anuria. A larger dose, namely, 5 mg. per kilogram, was found necessary to produce the latter result.

In cases of anuria from experimental mercuric chlorid poisoning, all attempts to reestablish the flow of urine by intravenous administrations of strongly diuretic solutions failed.

In animals that had received the minimum uniformly lethal dose of mercuric chlorid, but in which the kidneys were still capable of functioning actively, no success was attained in efforts to save the dogs by copious systemic lavage by means of intravenous injections of highly diuretic sugar or salt and alkali solutions. In fact, it usually shortened life. Lambert and Patterson[13] used diuretics both orally and by rectum, supplemented by diaphoretic measures in the treatment of clinical cases of mercuric chlorid poisoning. They attached considerable importance to the diuresis and sweating. However, they combined with these the administration of albumin in the form of milk, frequent washings of the stomach, and colonic flushing, so that it would be impossible to judge from their data how much, if at all, the sweating and diuresis in

13. Lambert, S. W., and Patterson, H. S.: Poisoning by Mercuric Chlorid and Its Treatment, Arch. Int. Med., November, 1915, p. 865.

themselves contributed to the success of their treatments. Moreover, none of the ten cases reported became anuric, which, in the light of the present experiments, would indicate that the quantity of mercuric chlorid that actually gained access to the tissues at large never in their cases greatly exceeded the minimum surely fatal dose, and might never have equaled it. The symptoms were those of a relatively mild grade of tissue poisoning, compatible with recovery without accelerated diuresis. It would appear as though the success of the treatment may have been due chiefly to the factors of delayed absorption and hastened elimination from the alimentary tract rather than the diuresis and sweating, although on the basis of the present experiments on dogs, no conclusion could be drawn as to the value of the sweating. Similar comments might be made concerning the method of treatment described by Weiss.[14]

Dogs that had received by vein the minimum uniformly lethal doses of mercuric chlorid died in spite of intravenous injections of sodium acetate-sodium phosphite solutions, even when the latter were given immediately after the poison had been given, and when the weights of sodium phosphite were from twenty to thirty-five times as great as the weights of the mercuric chlorid administered. The sodium phosphite-sodium acetate therapy has proved of no value in counteracting or preventing the deleterious effects of intravenously administered mercuric chlorid. Dogs and rabbits that had received by vein the minimum uniformly lethal dose of mercuric chlorid died in spite of the use of the best known forms of alimentary medication.

CONCLUSIONS

1. There is no sound experimental basis for the belief that the promotion of free diuresis contributes materially to the chances of recovery in mercuric chlorid poisoning, and this phase of treatment should not be permitted to obscure that which is more essential.

14. Solutions which are diuretic are not infrequently cathartic as well. This applies to sodium or potassium acetate, lactose, and to a very limited degree to the sodium chlorid-sodium carbonate solutions used by Weiss, even when the latter are given intravenously. The chief channel of elimination of mercury, as with the salts of heavy metals in general, is by way of the bowel, and a priori reasoning would lead to the conclusion that relatively little of these substances can be made to pass out by way of the urine under any circumstances.

2. Combined treatments which involve sweating, diuresis and increased elimination from the bowel probably owe their value chiefly to the latter effort.

3. It would appear in the light of the present study that when 4 mg. or more of mercuric chlorid per kilogram of body weight has entered the tissues at large, death regularly occurs, and that we have no adequate grounds for believing that death is preventable by any known form of treatment. Whereas, subsequent studies may add to our knowledge, it would appear that persons who have recovered from mercuric chlorid poisoning owe their lives to the fact that a lethal dose has never gained access to the extraportal circulation. Practical therapeutic efforts should be directed frankly toward the accomplishment of two things: (1) Mechanical removal of the poison from the lumen of the alimentary tract. (2) Antidoting the poison before it leaves the portal circuit, that is, particularly before absorption.[15]

15. Before the completion of the work described above a patient who had entered the Presbyterian Hospital on account of mercuric chlorid poisoning was treated at the request of Dr. Frank Billings, by means of intravenous injections of glucose and of hypertonic salt and alkali solutions (1.4 per cent. sodium chlorid in 0.3 per cent. sodium carbonate-Fischer), and also by renal decapsulation. The negative results of treatment paralleled those observed in animals. None of the methods restored a flow of urine, and the patient died on the seventh day.

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Treatment of Facial Erysipelas at Camp Cody, N. M.

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CAMP CODY, DEMING, N. M.

TREATMENT OF FACIAL ERYSIPELAS AT CAMP CODY, N. M.

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Between Nov. 6, 1917, and May 15, 1918, 104 cases of erysipelas were treated at the base hospital at Camp Cody. Most of these began on the face. The disease followed drainage of the mastoid in seven cases, abscess of the scalp in three cases, demonstrable ulcer in the nose in three cases, hordeolum in one case, and burns of the face in one case. The remaining ninety cases were called "idiopathic." On certain occasions an influx of such cases was noted after a period of high winds and dust storms, such as characterize this place and favor the development of inflammations of the mucous membranes of the head generally.

Although only one patient died as the result of erysipleas, the disease was responsible for a total of 2,497 hospital days, making it a matter of some importance to determine the best method of management and establishing it as a routine.

TREATMENT

Most of the methods known to the profession have been tested, including a sensitized streptococcus vaccine prepared here from the mastoid cases by Captain Lamb, chief of the laboratory service. Until about March 1, 1918, none of the methods tried showed any marked superiority over certain others; but at this time, Lieutenant Avata began a systematic employment of the method of collodion circumscription, first described in 1892 by Niehans. The results have

been striking. Before March 1, the cases treated by all other methods averaged 8.1 days of fever and 30.4 days in hospital. (The patients were discharged when redness, swelling and desquamation were all gone.) All patients treated after March 1 by collodion circumscription averaged 3.5 days of fever and 15 days in hospital, a cut of 50 per cent. in the number of days spent in hospital by the average case of erysipelas.

The method is not a new one, but it would appear that it is not so commonly used as it might be to advantage, and that frequently it is not carried out in such a way as to give the full results of which it

Fig. 1. Erysipelas beginning about the right eye. The disease having extended to the bridge of the nose, the collodion line is made to include the nose and both eyes.

Fig. 2. A case similar to that shown in Figure 1, front view.

is capable. The following details of the method as used at Camp Cody, by Lieutenant Avata, are, therefore, recorded.

Collodion, U. S. P. (nonflexible), is painted with a cotton swab or brush to form a stripe half an inch wide and from half an inch to an inch in advance of the line of induration, in such a way that the diseased area is completely circumscribed. The collodion stripe is painted over repeatedly until, when dry, it makes a deeply constricted furrow not broken or imperfect at any point. On the following days the collodion line is inspected for breaks, cracks or inadequate constriction at any point; if found, these are

repaired by further coats of collodion. When enough collodion is used to produce a continuous and sufficiently deep (from half to three quarters of an inch) linear constriction of the skin, the erysipelatous induration advances to the collodion, but not beyond it. The collodion is left in place until the temperature and swelling have wholly subsided. If there is a break in the collodion line or if the skin constriction is too shallow, the disease may pass through or under it. Presumably a successful result depends on a complete constriction of the lymphatics of the skin through which the infection travels.

Fig. 3.—Circumaural erysipelas; intense swelling, stopping abruptly at collodion line.

Fig. 4.—Method of sparing one eye when the disease has not yet involved the bridge of the nose.

Some ingenuity may be exercised in laying out the line on which the collodion is applied when dealing with erysipelas involving different parts and expanses of the face, head, neck, etc. In cases beginning about the ear it has been found feasible to clip the hair and encircle the ear; with unilateral involvements of the cheek or face which have already approached one eye but have not involved the nose, the line may be drawn up the nose between the eyes and around the lesion, leaving one eye within the circumscribed area, but saving the other, etc., as in the accompanying illustrations.

Within the circumscribed area the inflamed skin swells intensely, at first appearances more intensely

than in noncircumscribed cases; but this is mainly an appearance due to the contrast between the swollen area and the line of depression caused by the collodion, as may be noted in the illustrations. Patients have not complained more of discomfort or the sensation of tightness, when so treated, than those not circumscribed. On the contrary, the control patients complained that they were not given the benefit of circumscription. The latter do not become so toxic as those in which the disease is permitted to involve wide areas of skin (Table 2).

TABLE 1.—COMPARATIVE RESULTS OF THE TREATMENT

Total cases from Nov. 6, 1917, to May 15, 1918..... 104
Cases treated by collodion circumscription..... 23
Cases treated by all other methods..... 81

	Collodion Cases	All Others
Average stay in hospital	15 days	30.4 days
Average febrile period	3.5 days	8.1 days
Average maximum temperature 103	F.	104.5 F.
Incidence of complications	0.0 per cent.	15.3 per cent.

The collodion treatment is supplemented by the continuous application of cold compresses wet with a saturated solution of magnesium sulphate and by general measures, such as are rational in any febrile condition.

TABLE 2.—RESULTS IN FOUR CASES TREATED WITH AND FOUR WITHOUT COLLODION CIRCUMSCRIPTION

	Case No.	In Hospital, Days.	Febrile, Days.	Highest Temperature.	Highest White Blood Count.	Complications.
Circumscription Cases.	1	12	3	102.0		None
	2	20	3	104.4		None
	3	21	5	101.4	16,000	None
	4	22	2	99.4	8,000	None
	Average	18.75	3.25	101.8	12,000	None
Controls	1	33	7	104.6	.....	Abscess
	2	34	8	104.0	.....	Abscess
	3	16*	11	104.0	23,000	None
	4	22	14	105.4	22,000	None
	Average	26.25	10	104.5	22,500	40%

*This patient had erysipelas six days before admission to the hospital.

RESULTS

Of the twenty-three erysipelas patients treated by collodion circumscription, all but one entered the hospital after Feb. 1, 1918, whereas most of the others were admitted before that date and after Nov. 6, 1917. The question could be raised, therefore, as to whether the marked improvement in results was influenced by other factors besides the treatment, as

for instance, a natural decline in the virulence of the infecting organism, an increased resistance on the part of the seasoned troops, differing practices with reference to the date of release, etc. After the middle of April, to control this point, circumscription was employed only in alternate cases, while the controls were treated simply with cold compresses of saturated magnesium sulphate solution. The results are given in Table 2.

CONCLUSION

The method of treating erysipelas by collodion circumscription as described above has led to a marked reduction in the time spent in hospital by such patients. It also markedly shortens the length and the height of the fever curve, lessens the toxic symptoms generally, and in facial erysipelas reduces the frequency of abscess formation almost to nothing, since abscess seldom occurs with erysipelas confined to the face. No comparable results having been obtained by any other method, the collodion circumscription method has been established as a routine.

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THE EFFECT OF VARIATIONS IN THE AVAILABLE ALKALI ON THE YIELD OF ACETONE IN THE OXIDATION OF BUTYRIC ACID WITH HYDROGEN PEROXIDE.*

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This report concerns oxidations of butyric acid and acetone in the presence of varying quantities of alkali and of glucose, the experiments having been devised with reference to the problem of acidosis of the diabetic or starvation type and of so called antiketogenesis.

Dakin[1] showed that ammonium butyrate may be oxidized by hydrogen peroxide in faintly alkaline solutions to yield, along with products of oxidation at the α-carbon atom, other products; namely, acetone and probably acetoacetic and β-hydroxybutyric acids due to oxidations confined to the β-atom. Neubauer[2] made a similar observation using potassium persulfate as the oxidizing agent, whereas it is commonly held that oxidations of the fatty acids *in vitro* take place as a rule only at the α-atom. Thus under certain conditions butyric acid only gives proof of susceptibility to oxidative attack at the α-atom and under others at both the α- and β-atoms. It might be that the primary oxidative attack on the butyric acid molecule is the same in each case but that the evidences of β-oxidation are lost under the conditions of most laboratory oxidations. Another possibility is that the α- and β-atoms in butyric acid manifest varying degrees of susceptibility to oxidation under varying physical chemical condi-

* The work reported in this article was done in relation to the "Studies on the Theory of Diabetes" being carried out under the direction of R. T. Woodyatt in this laboratory.

[1] Dakin, H. D., *J. Biol. Chem.*, 1908, iv, 77.

[2] Neubauer, O., *Deutsch. Arch. klin. Med.*, 1908–09, xcv, 211.

tions. In the present experiments the attempt was made to ascertain whether the point of oxidation may be shifted by varying the conditions of the experiment. Attention was given chiefly to two variables—alkalinity and quantity of glucose—which were chosen because of the following biological consideration.

1. The so called acetone bodies are commonly assumed to arise in the organism from butyric acid by oxidations at the β-atom. Their occurrence in abnormal quantities in the blood and urine is accompanied by a state of acidosis and the question arises as to the relationships which exist between alkali deficits and abnormal accumulations of β-oxidation products of butyric acid in the body fluids.[3] It is therefore of interest to ascertain whether an alkali deficit is fundamentally favorable to the greater formation of acetone bodies from butyric acid.

2. In connection with the well known power of carbohydrate feedings to lessen or annul the acidosis of fasting, diabetes, etc., the question arises as to how glucose in the course of its metabolism prevents all outward expression at least of β-oxidations. Is the so called antiketogenic effect of carbohydrate the expression of a purely quantitative decrease of the fatty acid combustion, a "sparing" effect of fat by sugar, or is it due to a qualitative alteration of the type of fatty acid oxidation, such, for

[3] It is undeniable that alkali may be administered to individuals with ketosis until the urine is alkaline and the hydrogen ion concentration and the bicarbonate reserve of the blood plasma are normal and that ketosis may still be present without acidosis in the sense that many now use this term. This is true, but it does not follow because a normal reserve of bicarbonate is observed in the blood plasma taken as a whole that there is no deficit of alkali anywhere in the body. There may still be a shortage of effective alkali in the zones of chemical reaction within the cells where oxidations of fatty acids are going on.

In an earlier paper from this laboratory (Woodyatt, R. T., *J. Biol. Chem.*, 1915, xx, 129) emphasis was laid on the parallelism between the behavior of sugars in solutions of higher or lower alkalinity and their behavior in milder or more severe cases of diabetes, respectively. The work of Murlin and Kramer and their associates—showing apparently that alkali feedings may increase the utilization of sugar in diabetes—harmonizes with this view. Nor does the fact that alkali feedings do not increase carbohydrate utilization more than they do, constitute a valid objection to the conception that the normal utilization of glucose depends on alkali or its equivalent.

instance, as a shift in the center attack away from the β-atom and toward the α-atom? In either case is it, as some have supposed, a direct and purely chemical interaction between *oxidizing* glucose and oxidizing fatty acids, in which case it should be possible to duplicate it by oxidizing butyric acid and glucose simultaneously in the same solution? Or is it an effect which involves and depends upon changes in the cell architecture and one therefore which could not be duplicated *in vitro* in a simple homogeneous system? The experiments to be described were designed to give some light on these questions.

Owing to the great difficulty of analyzing quantitatively a mixture such as is formed in these oxidations, only the acetone was determined quantitatively. This was done on the basis of the assumption that the amount of acetone present is a relative index of the amount of other acetone bodies also present; *i.e.*, the amount of β-oxidation products present in the mixture.

EXPERIMENTAL.

Variations in the Alkalinity.

Experiment I. Action of Hydrogen Peroxide on Potassium Butyrate in the Presence of Variable Concentrations of Alkali.—4.4 gm. of butyric acid were weighed out in each of five flasks. They were all neutralized with potassium hydroxide. Additional potassium hydroxide was added as follows: to (1) none; to (2) 1.4 gm. or 0.5 molecule, to (3) 2.8 gm. or 1 molecule; to (4) 5.6 gm. or 2 molecules; to (5) 11.2 gm. or 4 molecules. They were all diluted to 387 cc., and 113 cc. of 3 per cent hydrogen peroxide were added (total volume 500 cc.), and the mixtures were allowed to stand at room temperature for 1 month. Even at the end of this time they all gave positive tests for hydrogen peroxide. 100 cc. of each of these solutions were distilled at ordinary pressure by heating the distilling flask with a ceresin bath, at a bath temperature of not over 160°. The distillate of (1) had a strong odor resembling acrolein or crotonic aldehyde. This odor diminished in the others and was absent in (5). These distillates were titrated for acetone by the Lieben iodoform method. From the amount of iodine solution that was consumed in each case the

amount of acetone that could have been present in each experiment was as follows: (1) 0.293 gm., (2) 0.0973 gm., (3) 0.0567 gm., (4) 0.0655 gm., (5) 0.0675 gm. In verifying these results the solutions were used up. In every case solid iodoform separated out and the odor was unmistakable.

Since in this series of experiments no effort was made to eliminate other compounds that form iodoform or that absorb iodine, another series was run. The same amounts of butyric acid and hydrogen peroxide were used. Each was diluted to a volume of 500 cc. The amounts of potassium hydroxide used were as follows: (1) no excess; (2) 0.125 molecule or 0.35 gm. in excess; (3) 0.25 molecule or 0.7 gm. excess; (4) 3.8 molecule or 1.05 gm. excess; (5) 0.5 molecule or 1.4 gm. excess; (6) 0.625 molecule or 1.75 gm. excess; (7) 0.75 molecule or 2.1 gm. excess; (8) 0.875 molecule or 2.45 gm. excess; (9) 1 molecule or 2.80 gm. excess; (10) 1.5 molecules or 4.2 gm. excess; (11) 2 molecules or 5.60 gm. excess; (12) 3 molecules or 8.4 gm. excess. These were distilled off as above after standing at room temperature 40 days. The amount of iodine consumed corresponded in (1) to 0.354 gm. of acetone, in (2) to 0.0764 gm. of acetone, in (3) to 0.0484 gm. of acetone, and in the remainder fluctuated a little but was about the same as in (3). Since in test experiments it had been found that when an acetaldehyde solution of known iodoform titer was heated with excess silver oxide in the presence of potassium hydroxide, in pressure flasks for 2 or 3 hours, it no longer absorbed iodine when titrated by the customary iodoform method, the above solutions were submitted to the same treatment. After the treatment was complete the silver and excess silver oxide were filtered out, the precipitate and filter were washed, and the filtrate and washings again distilled, because the silver salts interfered with the titrations. The acetone titrations obtained with these distillates corresponded in (1) to 0.161 to 0.175 gm. of acetone; in (2) to 0.030 gm. of acetone, and in the remainder of the experiments the values fluctuated between 0.030 and 0.040 gm. of acetone in each case, when calculated for the entire solution.

The results of both sets of experiments show that the yield of acetone was greatest in the flasks containing the least alkali and declined rapidly in the flasks containing more alkali.

Experiment II. The Effect of the Total Amount of Alkali on the Yield of Acetone in the Oxidation of Butyric Acid by Hydrogen Peroxide.—In some earlier experiments on the oxidation of glucose with potassium permanganate[4] in the presence of variable concentrations of alkali, it was found that there was a sharp change to a maximum yield of oxalic acid when the total amount of alkali present was sufficient to neutralize completely all of the acids produced by the oxidation. Since variations in the alkali concentrations of the body tissues must of necessity be small, but since the total available neutralizing power may easily vary in a much larger sense, it seemed desirable to repeat the oxidation of potassium butyrate under conditions in which the initial concentration of the alkali in each experiment is the same while the total amount varies. In each experiment the potassium salt of 4.4 gm. of butyric acid and 113 cc. of 3 per cent hydrogen peroxide were used. The experiments and amounts of acetone obtained were as follows:

	Total volume 0.01 N alkali.	Acetone.
	cc.	*gm.*
(1)	200	0.163
(2)	400	0.075
(3)	600	0.050
(4)	800	0.042
(5)	1,000	0.050
(6)	1,600	0.043
(7)	2,000	0.046
(8)	4,000	0.045

The above results are strictly comparable with the results obtained in Experiment I in which the concentration of the alkali was varied. There is no question, therefore, but that the amount of alkali available is a fundamental influence on the amount of acetone found in these experiments.

Critical Experiments.

It was thought that perhaps the acetone formed in the more alkaline flasks in Experiments I and II was being (*a*) condensed by the alkali and (*b*) being oxidized by the peroxide. (*a*) was tested first.

[4] Witzemann, E. J., *J. Am. Chem. Soc.*, 1916, xxxviii, 150.

(*a*) *Action of Alkali on Acetone.*—50 cc. of a titrated acetone solution containing 0.05 gm. of acetone were mixed with 20 cc. of 20 per cent potassium hydroxide solution and 80 cc. of water, making the solution 0.47 N alkali, and distilled. The distillate showed 0.05 gm. of acetone. 10 cc. of this 0.1 per cent acetone solution were added to each of three flasks containing 200 cc. of water, and 10, 20, and 30 cc. respectively, of 20 per cent potassium hydroxide were added. After standing 3 days at room temperature, all showed an identical titer of 1.0 gm. of acetone per liter for the acetone solution used.

These experiments showed that acetone is not condensed by alkali under the conditions used in these experiments.

Portions of this acetone solution were also subjected to the treatment with alkaline silver oxide. In all cases some of the acetone was lost, showing thus that the amount of acetone surviving this treatment will be smaller than the amount originally present.

In view of all the experimental work of other authors on the iodoform method for determining acetone and the experiments described in the preceding paragraphs, it is probable that the data obtained by direct titration of the distillates in oxidation experiments like I and II give a result that is too high when calculated entirely to acetone. Moreover, the titrations for acetone are probably too low after the treatment with silver oxide and alkali. But assuming that both series of results show the relative amounts of acetone present, it is clear that the amount of acetone present is larger the closer neutrality is approached. These experiments outwardly definitely verify the idea that a larger proportion of acetone bodies is formed in the more acid conditions.

This conclusion is modified by special experiments designed to test its truth, which are described under (*b*).

(*b*) *The Action of Hydrogen Peroxide on Acetone in Weakly Alkaline Solutions.* In some experiments on the oxidation of acetone with potassium permanganate in the presence of variable amounts of alkali[6] it was found that at low concentrations of alkali the acetone mixture (2 gm. of acetone in 1 liter of solution)

[6] Witzemann, *J. Am. Chem. Soc.*, 1917, xxxix, 2657.

came to equilibrium with unchanged acetone and oxidation could not be continued.[6]

It was of interest to know whether the same thing occurs when hydrogen peroxide is used. It was found that although hydrogen peroxide does not act so vigorously as permanganate under these conditions, it nevertheless qualitatively gives the same result.

50 cc. of an acetone solution containing about 2 gm. in 50 cc. were added to 900 cc. of a solution containing enough alkali for 1 liter, having the following concentrations: (1) blank, no alkali; (2) no alkali; (3) 0.01 N alkali; (4) 0.02 N alkali; (5) 0.03 N alkali; (6) 0.05 N alkali; (7) 0.10 N alkali. After standing 5 days at room temperature 5 cc. were pipetted off and titrated for acetone. Since the titration values were practically identical it was again shown that acetone is not condensed by these concentrations of alkali. 50 cc. of hydrogen peroxide (3.07 per cent) were then added to each flask except (1) to which 50 cc. of water were added. After remaining 13 days in a dark cupboard, hydrogen peroxide was tested for by the chromic acid-ether test. Only (2) showed the presence of hydrogen peroxide in this way.

5 cc. of each of the above solutions were pipetted off and placed in a flask with about 30 cc. of water. A pinch of manganese dioxide was added to decompose the hydrogen peroxide. Only two showed any evolution of oxygen. The mixtures were carefully filtered, the filters washed, and the filtrates titrated for acetone as usual.

In each experiment there were originally 2.11 gm. of acetone. The titrations showed a loss of 0.096 gm. or 4.8 per cent in (1); 0.096 gm. or 4.8 per cent in (2); 0.28 gm. or 13.2 per cent in (3); 0.30 gm. or 14.2 per cent in (4); 0.39 gm. or 18.5 per cent in (5); 0.46 gm. or 21.8 per cent in (6); 0.47 gm. or 22.2 per cent in (7). A careful restandardization of the iodine and thiosulfate solution showed that their titer was unchanged and the loss in (1) and (2) was presumably due to other causes than errors in titration. That this error was incurred during the treatment with manganese dioxide and the subsequent filtration was shown in another series of experiments in which the hydrogen peroxide was

[6] Sachs F., *Ber. chem. Ges.*, 1901, xxxiv, 497.

all decomposed without any treatment with manganese dioxide and in which the acetone content was found to be practically unchanged. If these solutions were then put through the manganese dioxide treatment the acetone loss was about 5 per cent. Thus if 4.8 per cent is subtracted from the above values, since presumably the evaporation of acetone was about the same in all cases, the values remaining would more truly represent the amount of acetone destroyed by hydrogen peroxide.

	per cent		*per cent*
(1)	0.0	(4)	9.4
(2)	0.0	(5)	13.5
(3)	8.4	(6)	17.0
		(7)	17.4

If 0.47 gm. of acetone in (7) was oxidized to one molecule of acetic acid plus one molecule of formic acid, then three atoms of oxygen, or 0.39 gm., or 0.83 gm. of hydrogen peroxide, would be required. Since 1.53 gm. of hydrogen peroxide were added in the above experiments it is clear that there was enough hydrogen peroxide present to oxidize nearly twice the maximum amount of acetone oxidized to acetic and formic acids. If the sole products were acetic and carbonic acids, 1.11 gm. of hydrogen peroxide would be required. It is thus clear that the amount of peroxide added could have produced the observed amount of oxidation. The nature of the products was not determined.

The above experiments were repeated a number of times under a variety of conditions designed to prevent loss of acetone by evaporation during the oxidation. This result was not realized perfectly in any complete series. Although the quantitative results varied somewhat in each case the general sense of the results was always the same. It would not be profitable to describe the individual results since the nature of the variations causing the isolated abnormal results is not known with certainty.

The above experiments show that acetone is more and more easily oxidized by hydrogen peroxide with increasing alkali concentrations. That all of the hydrogen peroxide is not used in oxidation of acetone is shown by the fact that it sometimes disappears from the solution (kept in the dark) without producing any noticeable oxidation. Thus perhaps the result in any experi-

ment is the resultant between the velocity of catalytic decomposition of the hydrogen peroxide and the velocity of oxidation of acetone by it. It will not be advisable at present to discuss the mechanism of the processes involved here.[7] It is sufficient to note that acetone in the presence of hydrogen peroxide and varying amounts of alkali comes to equilibrium with the system with an increasing stability of acetone with decreasing alkali concentration.

May acetone be oxidized by air? In connection with the above experiments the question arises as to whether all or part of the oxidation of acetone observed may not be due to the oxygen of the air. In order to answer this question about 1 gm. of acetone was made up to 500 cc. with enough alkali in each case to make the concentration as follows: (1) no alkali; (2) 0.01 N alkali; (3) 0.05 N alkali; (4) 0.10 N alkali; (5) 0.20 N alkali. 5 cc. of the mixtures were titrated for the acetone as usual. 1.04 gm. was the average acetone content. The flasks were stoppered tightly with rubber stoppers and were agitated vigorously at least once each day. After 12 days the average acetone content was 1.03 gm. After 1 month the average acetone content was found to be 1.03 gm. These experiments show that if atmospheric oxygen cooperates with hydrogen peroxide in the oxidation of acetone it does so at some lower stage in the oxidation than acetone.

These results of the experiments described under (*b*) prove that acetone formed in experiments like I and II is liable to be partly oxidized under the conditions of the experiment and that the amount of acetone found is not necessarily all that was formed.

Rigidly interpreted this result would indicate that we no longer have any conclusive evidence that a greater proportion of acetone is formed in the less alkaline flasks in Experiments I and II. In fact, a closer examination of the results in Experiments I, II, and (*b*) shows that in each case the amount of acetone oxidized in the corresponding experiment in (*b*) by less than half as much hydrogen peroxide is greater than the amount of acetone observed in the corresponding experiment in I and II. This result, however, does not in any sense invalidate the sug-

[7] The interrelation of these two processes is being investigated experimentally.

gestion that a greater proportion of acetone bodies is formed when butyric acid is oxidized in less alkaline conditions. It only shows that the value of this suggestion cannot be established in this way. The one positive result that comes from these experiments is the confirmation of the suggestion that acetone, and presumably the acetone bodies, is oxidized with greater difficulty the less alkaline the solution becomes when all other conditions are identical.

Variation of Glucose.

Experiments to Determine the Effect of the Presence of Glucose on the Yield of Acetone from a Constant Amount of Butyric Acid, Hydrogen Peroxide, and Alkali.—As was stated in the introduction, the presence of glucose undergoing oxidation has the effect of diminishing the ketosis under discussion. Can this effect be produced *in vitro*, and if so under what conditions? The following two series of experiments were done in order to get some experimental light on this subject.

Experiment III.—4.4 gm. of butyric acid in the form of the potassium salt in a total volume of 600 cc., containing enough potassium hydroxide to be 0.01 N at the beginning, and 113 cc. of 3 per cent hydrogen peroxide also contained glucose as follows: (1) no glucose; (2) 0.09 gm.; (3) 0.27 gm.; (4) 0.45 gm.; (5) 0.90 gm.; (6) 1.80 gm. After standing 1 month in the dark (1) was free from hydrogen peroxide, (2), (3), and (4) showed traces, and (5) and (6) gave strong tests for hydrogen peroxide. These solutions were analyzed for acetone in the usual way and even after treatment with alkaline silver oxide showed a gradually but markedly increasing acetone content with increasing glucose content. It was immediately suspected that the available alkali had been progressively consumed. Titrations showed that only (1) and (2) were still alkaline. The others were all distinctly acid in reaction. This result, although not exactly what was desired, is extremely interesting when taken in conjunction with the next series of experiments and will be emphasized later on.

Experiment IV.—The presence of variable amounts of glucose caused the amount of available alkali in turn to vary. In order to eliminate this additional variable as much as possible the experiments were again set up in the same way and small, known

portions were removed each day at first and then every few days, and titrated with 0.1 N sulfuric acid. The calculated amount of alkali was added each time to bring the alkali concentration up to its original value (0.01 N). The total volume of potassium hydroxide, 0.84 N, added to each flask in this way was as follows: (1) 10.97 cc.; (2) 11.97 cc.; (3) 13.44 cc.; (4) 17.87 cc.; (5) 21.76 cc.; (6) 26.02 cc. The amount of alkali thus consumed gives a relative index of the amount of acid production that occurs in these oxidations. Since the amount of potassium butyrate used was constant it is easy to ascribe the variable alkali consumption to the glucose oxidation. Owing to the fact that the mixture was constantly alkaline only a small amount of acetone was found even in Flask 1. In (2) still less was formed and in the remainder the amount was quite small and nearly constant as far as the data showed. In these experiments the amount of iodine consumed by the distillate before and after treatment with alkaline silver oxide was nearly identical. This is in marked contrast with the first series of experiments in which the iodine consumed by the untreated distillates increased with the sugar content and clearly indicates a marked difference in the final state of the compounds in the two solutions. The latter series of experiments gave the expected results and when taken in conjunction with the first series demonstrates clearly in another way the marked and fundamental importance of an adequate supply of alkali in such oxidations.

Experiment V. Effect of the Presence of Glucose on the Yield of Acetone from a Constant Amount of Butyric Acid and Hydrogen Peroxide in the Presence of So Called Regulator Mixtures.—There seems to be a feeling that the source of alkalinity influences oxidation. Thus in order to put the above experiments on a basis that would be more readily acknowledged to resemble biological conditions they were repeated in the presence of so called regulators. Preliminary experiments showed that calcium carbonate, calcium hydroxide, and sodium carbonate are not available for these experiments since they so rapidly catalyze the decomposition of hydrogen peroxide. It was therefore necessary to use the phosphate mixture. In these experiments it was desired to use enough hydrogen peroxide to oxidize both the butyric acid and glucose used to carbon dioxide. Since acetone itself is gradually oxidized by peroxide the oxidation was stopped after a time before the peroxide had all been reduced.

The experiments were conducted as follows: The amounts of K_2HPO_4 and KH_2PO_4 given in the first two columns of the table were dissolved in 50 cc. of water, the solution was cooled, and 0.25 gm. of butyric acid as the potassium salt (5 cc. of a 5 per cent solution) was added. 0.25 gm. of glucose (0.5 molecule) was also added to the second flask. 50 cc. of 3 per cent hydrogen peroxide were then added to both. In Experiments 7 and 8 the salts were dissolved in 150 cc. of water. After standing at room temperature for 24 hrs. stoppered with a rubber stopper, the entire mixture was transferred to the distilling flask and after adding some precipitated calcium carbonate to facilitate the decomposition of excess hydrogen peroxide and some broken porcelain to facilitate boiling, the acetone was distilled as usual. The results of such experiments should be expected to vary somewhat but in general they tend to run parallel with the results obtained above, using potassium hydroxide. In Experiments 1 to 6 more acetone is obtained at first in the presence of glucose than in its absence but as the available alkali is increased (K_2HPO_4) this advantage diminishes and in the later experiments the acetone production is about equal in the presence and absence of glucose. The use of larger amounts of K_2HPO_4 without changing the proportions, as in Experiments 7 and 8, likewise tends to equalize the acetone production in the presence and absence of glucose.

Experiment No.	K_2HPO_4	KH_2PO_4	With glucose.		Without glucose.	
			Total acetone.	Acetone after Ag_2O+KOH.	Total acetone.	Acetone after Ag_2O+KOH.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	5.35	6.92	0.096	0.0264	0.057	0.0135
2	6.92	5.35	0.089	0.0154	0.040	None.
3	6.92	5.35	0.081	0.0198	0.058	0.011
4	7.92	4.35	0.074	0.0133	0.112*	0.024
5	7.92	4.35	0.077	0.0197	0.075	0.019
6	8.92	3.35	0.075	0.0202	0.087	0.022
7	20.76	16.05	0.081	0.0248	0.075	0.026
8	20.76	16.05	0.086	0.0264	0.085	0.025

* Boiled dry in distillation.

Discussion of Experiments III, IV, and V.

Owing to the well known relationships of glucose oxidation to the formation of the acetone bodies in fasting and diabetes, it was important to observe the effect of the presence of variable amounts of glucose on the yield of acetone from a constant amount of butyric acid, hydrogen peroxide, and alkali. It was found in Experiment III that the products of glucose oxidation caused a progressive diminution in the alkalinity and accordingly an increasing production of acetone with increasing amounts of glucose. That is, when an inadequate supply of alkali is present in the presence of sufficient oxidizing agent and increasing quantities of glucose, there is an increased production of acetone. In this series glucose not only does not act antiketogenically but seems to favor ketogenesis. In Experiment IV in which the alkali supply was maintained the acetone production was at a minimum in all but those flasks in which there was little or no sugar present. In Experiment III glucose favored ketogenesis; in Experiment IV it was antiketogenic. The reversal in its effect was due to the control of the alkalinity. Experiment IV gives *in vitro* confirmation for the idea that the sugar spares[8] the oxidation of fats in the presence of adequate alkali supplies. The mechanism by which this may be effected is suggested by the result of Experiment VI.

[8] The word "spares" in this paper is apparently not used in the same sense as it has previously been used in this connection by other writers. It is used here in a figurative sense as explained below. According to the coexistence principle (see Ostwald, *Lehrb. allg. Chem.*, 1896–1902, ii, 244, 307) two reactions taking place in the same medium simultaneously proceed independently. When, however, both reactions are taking place at the expense of a single common reagent (in this case the oxidizing agent), not present in enormous excess, the velocity of each reaction from moment to moment depends on the velocity of the other reaction during the preceding period. If one reaction is using oxygen very rapidly the amount available for the other is more rapidly diminished. If one reaction is markedly stimulated with respect to the other for any given set of conditions, the compound being oxidized by the slower reaction may be said to be spared. Thus sparing in this sense is a chemical conception and contains no hint of a vitalistic point of view. This conception of sparing is involved in Experiments III, IV, V, and VI. More definite experiments on this subject are already partly completed.

Experiment V was a combination of all the preceding experiments in which mixtures of mono- and dibasic potassium phosphate were used instead of alkali. Since mixtures of these salts constitute an important part of the mechanism by which the reaction of the body fluids is regulated, it was thought that results obtained with such systems would be more readily acceptable. The general sense of the results confirms all that had been determined by the previous experiments, both as to the effect of available alkali on butyric acid oxidation and on the effect of glucose on this oxidation, without giving rise to any new conclusions.

Variation of Acidity.

The preceding experiments have shown that the less alkaline the system the greater the amount of acetone that was recovered from the experiment. It was of interest to know whether the amount of acetone that can be recovered continues to increase with increasing acidity. A few simple experiments were done to test this possibility, since there are theoretical reasons why it should be expected to be so.

Experiment VI.—The experiments were set up as follows: Each flask contained 50 cc. of 3 per cent hydrogen peroxide, and potassium butyrate and butyric acid (measured as standardized solutions) as follows: (1) 0.25 gm. of potassium butyrate; (2) 0.20 gm. of potassium butyrate and 0.05 gm. of butyric acid; (3) 0.15 gm. of potassium butyrate and 0.10 gm. of butyric acid; (4) 0.25 gm. of butyric acid. The total volume in each case was 100 cc. After standing at room temperature 42 hours, excess powdered manganese dioxide was added to decompose the large amount of unchanged hydrogen peroxide. When this decomposition, which was carried out in the distilling flask connected as for distillation, was complete, the entire solution was distilled and titrated for acetone as usual. The iodine consumed corresponded to about 0.001 gm. of acetone in each case. The total variation for the series did not exceed 10 per cent of the amount of acetone found. No solid iodoform was separated in these experiments but the odor of iodoform was unmistakable.

The results of this experiment indicate that the oxidation is so slow under these conditions that a difference in the amount of

acetone obtained is not clearly demonstrable after standing 42 hours, or else that there is no demonstrable difference. Since such experiments in the light of the above critical experiments could not give a satisfactory answer to the question as to whether there is an increase in the ratio of acetone formed, these experiments in acid conditions were not extended.

Similar experiments in which four molecules of sulfuric and hydrochloric acids were used for each molecule of potassium butyrate gave no striking increase in the yield of acetone, but with phosphoric acid an unexpectedly marked increase in acetone was observed.

This observation when taken in conjunction with the large acetone yields obtained with the phosphate mixtures in Experiment V opened up this problem from another angle. The experiments developed in this connection will be described in another paper.

Oxidation of Acetone and Glucose.

Experiment VII. Simultaneous Oxidation of Acetone and Glucose with Potassium Permanganate in the Presence of Varying Concentrations of Alkali.—Hydrogen peroxide presents one grave disadvantage as a quantitative oxidizing agent in alkaline systems owing to the fact that it is catalytically decomposed by alkali. Since the velocity of this decomposition varies with the composition of the system no quantitative results on the amount of oxygen consumed can be obtained in this way. Therefore in any study of the sparing effect of one metabolite upon another some other oxidizing agent in which this spontaneous decomposition is absent or negligible must be used. Since acetone may be regarded as one end-member of the β-oxidation of butyric acid which is difficult to oxidize, it was of interest to learn to what extent glucose spares the oxidation of acetone in systems containing an equal amount of both but a variable amount of alkali. Ten experiments were done as follows: 0.5 gm. of acetone, 0.5 gm. of glucose, and 4.0 gm. of potassium permanganate (insufficient for complete oxidation of either the glucose or acetone alone) were placed in 500 cc. of a solution containing alkali as follows:

		Acetone.	
		gm.	*per cent*
(1)	No alkali nor $KMnO_4$ (blank)	0.547	100.0
(2)	" "	0.403	73.0
(3)	0.6 gm. KOH (0.021 N)	0.326	59.6
(4)	1.21 " " (0.043 ")	0.261	47.7
(5)	1.82 " " (0.064 ")	0.237	43.3
(6)	2.43 " " (0.087 ")	0.219	40.0
(7)	3.03 " " (0.108 ")	0.218	39.8
(8)	6.06 " " (0.216 ")	0.194	35.4
(9)	19.44 " " (0.694 ")	0.200	36.5
(10)	38.88 " " (1.388 ")	0.185	33.8

After reduction was complete, *i.e.* after all the permanganate was reduced to manganese dioxide, the acetone content was determined in the usual way. The results are given in the last column. The data show that two and one half times as much acetone was oxidized in Flask 10 as in Flask 2 and that the transition is gradual. From these experiments it may be concluded that the capacity of glucose to spare acetone from oxidation diminishes with increasing alkalinity, or conversely the capacity of acetone to spare glucose diminishes with decreasing alkalinity. Or in physical chemical terms, the velocity of oxidation of acetone increases rapidly with increasing alkalinity even in the presence of glucose.

But not only does the velocity of oxidation of acetone with respect to glucose in the same system vary but also the velocity of permanganate reduction as a whole varies markedly from one experiment to the other. The velocity increases with rising alkalinity. In the more alkaline experiments it becomes so rapid as to be apparently instantaneous. In the case of Flask 2 several days were required for complete reduction.[9]

This experiment gives further illustration of the complexity of the processes involved in oxidation.

DISCUSSION.

Experiments I and II indicate that when butyric acid is oxidized by means of hydrogen peroxide in dilute solution in the presence of variable amounts of alkali the absolute quantity of

[9] The above is simply a preliminary account of one result in a more comprehensive study of this question that is under way in this laboratory.

acetone recoverable is greatest when the reaction of the system is neutral to faintly acid. The preliminary data mentioned in connection with Experiment VI indicate that the nature of this acidity has some influence on the yield of acetone. The important question as to the exact conditions that promote a maximum yield of acetone will not be discussed until the effect of other variables has been more fully studied.

The yield of acetone falls as the alkalinity increases notwithstanding an increase in the velocity of the oxidation of butyric acid. This decrease of the acetone yield is explainable in part if not wholly by the fact that acetone itself becomes increasingly susceptible to oxidation with the increasing alkalinity, but it was not proved that this phenomenon accounts for the entire decrease of the acetone yield. The original question as to whether the point of primary oxidative attack may be shifted by changing the reactions of the medium remains unanswered.

In studying the effect of the presence of glucose it was found that oxidizing glucose may change the reaction of the medium and so influence the acetone yield. But we have found nothing to indicate that the oxidation of glucose simultaneously with butyric acid influences the nature of the products formed from the latter.

On the other hand, Experiments III, IV, and V demonstrate how glucose may "spare" butyric acid in a manner which affords an interesting chemical analogy to the so called "sparing effect" of carbohydrate in the body.

Experiment VII gives a graphic illustration of how this "sparing" effect too may vary with changes in the reaction.

It will not be advisable to discuss the above results at any greater length but a few more general statements should be made. In any such complicated process as the oxidation of butyric acid or acetone there are many possible courses of reaction through many intermediate stages. The velocity of each of these reactions is influenced by minute changes in conditions. Some are more sensitive than others. In order to understand thoroughly the net result in such a case it would be necessary to know the constants for each set of conditions for each reaction. But since in biological conditions these reactions are carried out in heterogeneous systems devised to prevent the attainment of complete

equilibrium in life, such constants worked out in homogeneous systems *in vitro* might only have qualitative or no significance in the biological conditions. Nevertheless it seems certain that the experiments described here represent a constructive step toward a solution of this problem.

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ASCENDING INFECTION OF THE URINARY TRACT

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The exact route by which an infection travels from the bladder to the ureters, pelvis and kidney substance is still an open question. The routes by which this infection may take place are: (1) By way of the bladder lymphatics to the lymphatics of the ureter and kidney; (2) by direct extension through the wall of the bladder and the ureter; (3) by direct extension up the lumen of the ureter, and (4) by way of the blood stream.

A brief review of the literature shows that a single injection of bacteria into the bladder rarely leads to infection of the upper urinary tract unless there is some injury to the bladder. Kretchmer[1] has shown in some observations on the bladder of children that regurgitation of the bladder contents into the ureter does occasionally take place. Bauereisen[2] has shown both histologically and experimentally that in tuberculosis, at least, the production of an infection by direct extension through the wall of the bladder and ureter is a possibility. A great deal of work has been done on the lymphatics of the bladder, ureters and kidneys, showing very definite anastomosis between the different portions of the bladder, ureters and kidneys. Furthermore, anastomosis between the peri-ureteral lymphatics and those of the submucosa and muscularis has been demonstrated. Sweet and Stewart,[3] in their work on the transplantation of ureters into the intestinal wall, were able to demonstrate that the infection travels up the ureteral lymphatics to the kidneys. Their work, however, does not prove that urinary tract infection through the lumen of the ureter may

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1. Kretchmer: Cystography, Surg., Gynec. & Obst. **22**:209, 1917.

2. Bauereisen: Beitrag zur Frage der Aszendierenden Nieren-tuberculose, Ztschr. f. Gynäk. u. Urol. **2**:132, 1910. Idem.: Ueber die Lymph gefaesse des Menschlichen Ureters, Arch. f. Gynäk. u. Urol. **2**:235, 1910. Idem.: Ztschr. f. Gynäk. u. Urol. **4**:124 (1913).

3. Sweet and Stewart: Ascending Infection of the Kidneys, Surg., Gynec. & Obst. **18**:460, 1914.

not occur without involvement of the lymphatics. David,[4] in his article on ascending urinary infections, calls particular attention to the fact that peri-ureteral and pelvic infiltration is not synonymous with infection of the urinary tract. This applied particularly to the work of Eidrendrath and Kahn and Eisendrath and Schultz.[5] They demonstrate periureteral infiltration but no involvement or inflammatory reaction within the pelvis or the presence of bacteria there. Cabot and Crabstre,[6] in a series of cases, were able to demonstrate the presence of colon bacilli in the blood before the acute onset of a pyelitis.

As David rightly points out, a good deal of work has been done without being properly controlled. The bacteriology of the urinary tract previous to experimentation has not been studied. The same is true of postmortem examinations in which the bacteriologic work is very scanty. Inasmuch as the work of David is perhaps the most complete and best controlled contribution on the subject, I will go at some length into a discussion of this work. His experiments were controlled by removal of a portion of the ureter just prior to the establishment of the experimental conditions, and urinary cultures were made in all cases. The technic employed was as follows: By incision through the abdominal wall, the right ureter was divided about 1 inch above the bladder. A piece was removed and the proximal stump of the ureter ligated. The bladder was then injected, either through the distal ureteral stump or by means of a needle through the wall of the bladder. The point of injection was then closed with a pursestring suture.

In seven dogs having unobstructed, nontraumatized bladders two had positive cultures in the bladder, left ureter and pelvis, with absence of any cellular infiltration of any of the tissues, and with sterile blood and sterile right hydronephrosis. In three dogs of the same series, in which there was a gross fibrinous exudate on the peritoneal surface of the bladder, the culture from the left ureter, left pelvis and blood were negative. There was, however, peri-ureteral infiltration, diminishing as the pelvis was approached. On the right side in all three of these

4. David: Ascending Urinary Infections, Surg., Gynec. & Obst., **26**:159, 1918.

5. Eisendrath and Kahn: The Rôle of the Lymphatics in Ascending Renal Infection, J. A. M. A. **66**:561, 1916. Eisendrath and Schultz: The Path of Involvement in Ascending Infection of the Urinary Tract, J. Med. Research **25**:295.

6. Cabot and Crabtree: Nontuberculous Renal Infections, Surg., Gynec. & Obst. **23**:495, 1916.

cases, despite a defect in the ureter, a definite infiltration with polymorphonuclear cells was found which became most intense in the fat about the pelvis. In only one of these three was the lumen of the pelvis infected, however. These five experiments show first of all that peri-ureteral and peripelvic infiltration does not of necessity mean infection of the lumen of the pelvis and ureter, and, secondly, the first two experiments prove that infection may travel by way of the ureteral lumen from the bladder to the pelvis.

To sum up the experiments of David, those with an unmolested flow, as well as those with a partially checked flow, it may be said that in eleven experiments on the right side in which a portion of the ureter was removed there was peri-ureteral and peripelvic infiltration with sterile urine in ten out of eleven cases. On the left side in which the urine was intact, there was peri-ureteral and subpelvic exudate in only four instances, but there were positive cultures of colon bacilli grown from the left ureter and left pelvis of the kidney in ten out of eleven experiments. With the controls used by David from the blood stream and cultures from the right hydronephrosis, the conclusion that he deduces, namely, that infection on the left side ascends from the bladder to the pelvis by way of the lumen of the ureter, seems to be justified. These experiments prove that in the experimental animal, at any rate, infection can travel by way of the lumen of the ureter from the bladder to the pelvis of the kidney.

The work that I wish to report consists of a series of rabbits that were infected by the injection of a pure culture of colon bacillus, isolated from a spontaneous case of pyelitis in the rabbit. The technic was as follows: The animals were catheterized, and after catheterization 5 c.c. of a broth culture of colon bacilli were allowed to flow into the bladder by gravity. The urine was controlled microscopically and by culture and only those animals were used that showed negative urine. In a series of 32 intracystic injections, 15 of the animals showed definite pyelitis. Of this entire series of animals, only one showed abscesses in the kidney. These were in small groups in both kidneys, differing thereby from the abscesses that are obtained by intravenous injection, which are usually diffusely scattered throughout the kidney substance. The presence of a pyelitis was controlled both bacteriologically and histologically. The gross appearance was usually about as follows: On opening the peritoneal cavity the bladder was usually only very slightly distended with urine. The peritoneum about the

bladder was edematous. The edema frequently extended up a short distance on the ureter. In a few instances there was a slight swelling of the ureter and an injection of the vessels of the ureter. In the marked cases of pyelitis the pelvis was definitely distended with pus.

The histology of the organs was roughly as follows: Bladder: There was usually a definite marked acute inflammatory reaction in the submucosa and, in many instances, extending throughout the thickness of the bladder wall. So much can be said for all of the animals. The histology of the ureters and kidneys can be roughly grouped into two classes: those in which there are definite evidences of ureteral and peri-ureteral infiltration and infection of the pelvis, and second, those in which the findings in the ureters and kidneys were practically only pus in the pelvis of the kidney. In the first group there were five instances and in the second ten.

In all probability there were two routes by which the infection of the pelvis was accomplished: (1) by way of the lymphatics; (2) by direct extension up the lumen of the ureter. From the work of David it of course is difficult to say whether we might not have the pyelitis develop by direct extension up the lumen and have at the same time peri-ureteral inflammation. The extent of this inflammatory reaction, differing very markedly from that described by Eisendrath and Schultz, in that it was always of polymorphonuclear character, makes it seem probable that in those instances in which the acute infiltration extended to the lumen of the pelvis the infection traveled by way of the lymphatics rather than by way of the lumen.

As a control, the kidneys of a rabbit that were intravenously injected were studied to see what the relationship of the pelvic and peri-ureteral inflammation was. The inflammation in practically all of these was limited to the papilla side of the pelvis, and in no instance did we find such gross inflammatory reactions about the parietal wall of the pelvis as was the case in the intracystic injections.

To summarize:

1. With intracystic injection in fifteen out of thirty-two cases, pyelitis was produced in the rabbit.

2. The histology of the lesions would indicate that: (*a*) the infection travels by way of the lymphatics from the bladder to the ureter to the kidney; (*b*) in other instances it may probably travel by way of the lumen of the ureter.

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EXPERIMENTAL PYELITIS IN THE RABBIT

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From a study of the literature as well as from our own experiments carried on with organisms isolated from human pyelitis cases, it is evident that only very occasionally do organisms localize in the kidney after intravenous injection unless there is some obstruction in the course of the urinary tract. The experiment of Hess (1), of Cabot and Crabtree (2) and of many others show that bacteria pass through the kidney in large numbers without affecting that organ. From the results of our experiments with colon bacilli isolated from human pyelitis cases, we had come to practically the same conclusion. In striking contrast to all this work are the results of this experimental study, in which infections of the kidney were produced after intravenous injections of the organisms in about two-thirds of the animals.

In like manner a study of the literature concerning ascending infection of the urinary tract, other than tuberculous shows that without some accessory factor it was practically impossible to produce an ascending infection of the kidney. Hess (1) in his experiments was able to produce an ascending infection by injecting turpentine into the bladder before injecting it with colon bacilli. Sweet and Stewart (3) showed that when the ureters were implanted into the intestinal wall, an ascending infection of the kidney occurred. Cabot and Crabtree (2) are inclined to the opinion that even when colon bacilli are carried in the lymph stream from the bladder, they reach the kidneys by way of the blood stream rather than by way of the lymph channels.

Eisendrath and Schultz (4) obtained positive cultures from the kidney in a number of their experiments but never macroscopic changes in the kidneys indicative of involvement of the

pelvis or substance of the kidney. In marked contrast to these observations are the results of our experiments. In 10 out of 15 intravesical injections we were able to demonstrate definite macroscopic lesions of the kidneys. In all of the experiments the urine was previously controlled by culture and by microscopic examination.

The results in these two series of experiments are so at variance with the work of this kind reported in the literature that we wish to report in detail the findings of all our experiments. The histological study of this large series of rabbit kidneys has shown us as others have previously observed, that the rabbit is subject to spontaneous kidney lesions in no small number of cases. In 10 of the animals studied, we were able to demonstrate lesions of the kidney that were definitely of longer standing than could result from our injections. Of the 10 animals showing chronic changes, 4 (rabbits 76, 63, 68 and 42) presented also acute lesions, from which colon bacilli were isolated, so that there can be little question that there was a double process in these 4 animals. In the other 6 (rabbits 31, 32, 40, 53, 57 and 87) only the chronic processes were observed. These chronic changes were manifested by focal increase in connective tissue and in small areas of lymphocytic infiltration. In some cases the infiltration left very little of the kidney substance. In others there was a more diffuse increase in connective tissue between the tubules.

In this connection it might be mentioned that these focal changes in the renal cortex gave rise to a mistake on our part in the number of cortical kidney abscesses, as reported in our previous communication. We found that 3 instances which we had supposed were acute abscesses proved on microscopic examination to be focal infiltrations with connective tissue and collections of lymphocytes. In a continuation of our work we have several times been struck by the difficulty in differentiating these focal infiltrations from abscesses macroscopically.

In some previous work (5) in which we attempted to produce kidney lesions in the rabbit with colon bacilli isolated from human pyelocystitis cases, we were able to obtain lesions in the

kidney in only about 12 per cent of the animals injected. It was during the course of these experiments, while controlling the urine in a series of animals before injection, that we examined a female rabbit that constantly had a large number of pus cells in the urine. Cultures taken from a catheterized specimen of urine on several occasions showed always a pure culture of a Gram-negative bacillus that had the cultural characteristics of Bacillus coli communior.

The organism had the following characteristics: It grew well on all media. There was a profuse yellowish growth on agar. Gelatin was not liquefied. Litmus milk was rendered acid and coagulated; but there was no digestion of the casein. Indol was produced. There was acid and gas formation in dextrose, lactose and saccharose.

As this bacillus formed the basis of this entire series of experiments, we give with some detail the following history of the animal:

February 7, 1917. The first urine examined showed about 150 pus cells to a low power field.

February 10 to February 20. Daily examination revealed a large number of pus cells, single and in groups.

February 20. A catheterized specimen of urine was cultured in litmus lactose agar, plain agar and broth.

February 21. There were innumerable colonies on both plates.

February 23. Specimens were expressed every other day showing albumin and casts in addition to pus cells.

March 3. Another catheterized specimen was cultured; pure culture, same as obtained on February 20.

The animal was chloroformed, as it was getting very much weaker.

Necropsy. Upon opening the peritoneal cavity the abdominal organs all appear practically normal. The bladder is distended with urine. The stomach, gastro-intestinal canal, spleen, liver, gall-bladder, heart and lungs show nothing abnormal. On dissecting free the overlying organs the ureters on both sides are seen to be considerably dilated as far down as the brim of the pelvis. Beyond this point they appear to be of normal diameter. No apparent pathologic constriction in the ureter at the brim of the pelvis visible. The pelvis of both kidneys are smooth and of a reddish brown color. The capsule strips

readily. On section the cortex is of normal thickness, the striations regular. The outer portion of the medulla is deeply congested. The pelves of both kidneys are markedly distended. The left kidney pelvis is filled with a thin purulent material, the right practically empty. The bladder urine show only relatively few leucocytes, numerous casts and much débris. Cultures taken from the bladder and right kidney pelvis yield a pure culture of Gram-negative bacillus.

Histology. The bladder mucous membrane in the entire section is practically intact. In areas there are small clusters of lymphocytes; there are practically no polymorphonuclear leucocytes to be seen in the section. Ureter: A series of 12 blocks taken from the ureter show only a distended lumen without any infiltration, acute or chronic. Kidney: There is considerable degeneration of the epithelium and dilation of the collecting tubules; in areas focally there is considerable increase in connective tissue. In the medulla, especially toward the tip of the papilla, there is marked leucocytic infiltration about the tubules. Eosinophil cells are much in evidence. The mucosa of the pelvis is apparently thickened and shows considerable infiltration with leucocytes (fig. 1). The pelvis is filled with masses of polymorphonuclear leucocytes.

EXPERIMENTAL TECHNIQUE

The technique used in the experiments was as follows: The intravenous injections were made with a bacterial suspension made by centrifuging 15 cc. of a twenty-four hour broth culture and suspending the bacilli in 5 cc. of physiologic sodium chlorid solution. One cubic centimeter of this suspension was injected into the ear vein. The intravesical injections were made after carefully catheterizing the animals and allowing 5 cc. of the bacterial suspension to run into the bladder. In the feeding experiments 8 cc. were introduced into the stomach by means of a tube. In the rectal injections 8 cc. of bacterial suspension were introduced as high as possible through a rectal tube.

In the intravenous series of experiments 31 animals were injected. The first 10 animals received 3 injections at intervals of two days. The remaining 22 received only a single injection of 1 cc. of a twenty-four hour culture of the organism. Of the 31 animals injected, 17 showed a definite pyelitis. This pyelitis

was unilateral in 5 instances and bilateral in 12. In 9 instances there were cortical abscesses in addition and in 1 instance medullary abscesses. Only 1 of the 5 cases in which the pyelitis was unilateral were there any abscesses in the cortex. The lesions in the pelvis usually consisted of a purulent exudate

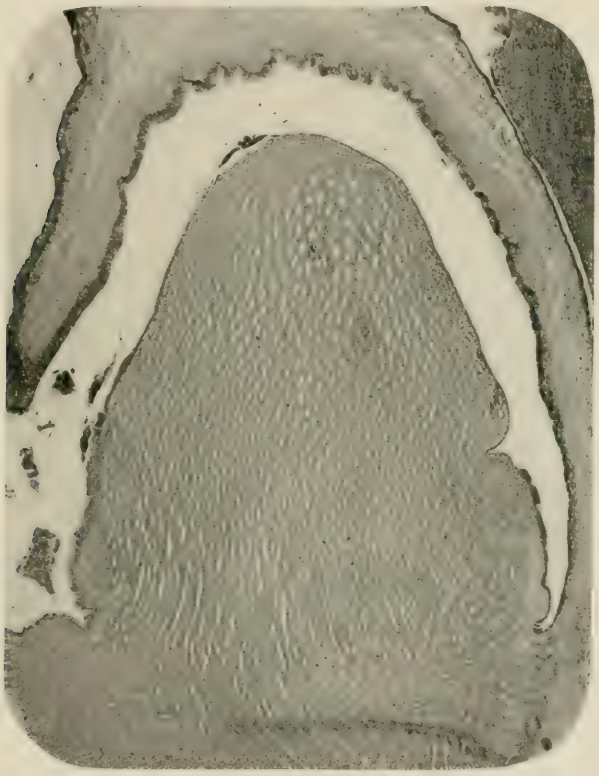

FIG. 1. SECTION THROUGH RABBIT PELVIS SHOWING COLLECTIONS OF LEUCOCYTES AND THE THICKENED MUCOSA

within the lumen and submucous infiltration with pus cells just beneath the mucous lining of the papilla. This submucous infiltration which in the milder cases involved only the papilla but in the more severe types extended also to the parietal lining of the pelvis, was probably the most typical lesion observed

TABLE 1

Intravenous injections

ANIMAL	MICRO-ORGANISMS FROM	DATE OF INJECTION	DATE OF DEATH	KIDNEY			CYSTITIS	URINE		REMARKS
				Pyelitis	Abscess	B. coli		Pus.	B. coli	
R18	R11	February 23	March 23	0	0	0	0	*	0	
R19	R11	February 23	March 29	Unilateral	0	0	0	*	*	
R20	R11	February 23	March 19	0	0	0	—	*	0	Abscess of hind leg
R21	R11	February 23	March 16	0	0	0	*	*	*	Small hemorrhage of cortex
R22	R11	March 8	March 10	0	0	*	0	0	*	
R23	R11	March 7	March 15	0	0	R * L0	—	0	*	
R24	R11	March 7	March 29	0	0	0	0	0	*	
R25	R11	March 8	March 29	0	0	0	0	0	0	
R26	R11	March 8	March 15	Bilateral.	Medullary	*	0	*	*	
R27	R11	March 8	March 16	Bilateral	Cortical	*	*	*	*	
R36	R26	March 17	March 21	Bilateral	Cortical	*	—	*	*	
R37	R26	March 17	March 23	Unilateral	0	—	—	*	*	
R40	R27	March 19	March 21	0	0	0	—	0	0	Diphtheritic colitis.
R41	R27	March 19	March 29	Bilateral	0	*	—	*	*	
R42	R27	March 19	March 23	Bilateral	Cortical	*	—	*	*	Broncho-pneumonia
R45	R36	March 22	February 28	0	Cortical	*		0	*	
R46	R36	March 22	March 25	Bilateral	Cortical	*		*	*	
R60	R42	March 29	April 2	0	0			0	0	
R61	R42	March 29	March 31	Bilateral	Cortical	—	0	0	*	
R62	R42	March 29	April 3	Bilateral	Cortical	R *	0	*	*	
R63	R42	March 29	March 31	Unilateral	0		0	0	*	
R64	R42	March 29	April 6	Bilateral		*	—	*	*	
R75	R41	March 31	April 15	Unilateral	Cortical	*	0	*	*	Abscess in groin—Bac. coli

R76	R41	March 31	April 2	0	Cortical	✱	—	0	✱	Chronic nephritis
R81	R64	April 9	May 1	Unilateral	0	✱	—	✱	✱	Ureters injected. Bladder culture. Mixed staphylococcus and B. coli
R82	R64	April 9	April 16	0	0	0	0	0	✱	Bladder culture. Mixed staphylococcus and B. coli. General peritonitis
R83	R47	April 9	April 12	Bilateral	Cortical			0	✱	
R84	R47	April 9	April 19	0	Cortical	0	0	0		
R89	R41	April 22	April 30	Bilateral	0		0			
R90	R41	April 22	April 30	Bilateral	L Cortical			✱		
R91	R41	April 22	April 30	0	0	0	0	0	0	
Total, 31				17	13					

in this group of cases. This was particularly striking in several instances, in which the purulent exudate during the course of examination had been washed out of the pelvis and practically the only lesion to be observed on histological examination was this thin zone of leucocytic infiltration just beneath the mucosa of the papilla. In the more severe cases there was a leucocytic zone of infiltration of considerable thickness below the mucosa. In the less severe cases only scattered polymorphonuclear leucocytes were seen below the mucosa. Pus cells wandering through the mucosa were relatively infrequent.

In those cases in which the pyelitis was complicated by cortical abscesses, the purulent material could usually be traced through the medulla into the pelvis. Whether a large series of section taken through this part of the kidney would have shown miliary abscesses in the cortex or medulla is a question that would be difficult to answer. In a number of animals several blocks were studied and in only one instance were abscesses found that had not been discovered macroscopically. This instance was not included in this series of experiments, because we have not included any animals dying within the first twenty-four hours. The abscess formation was characterized by the collection of pus cells within the glomeruli. In the section studied there was nothing found to suggest that the bacteria were carried directly to the capillaries lining the pelvic wall, rather than that they were excreted through the glomeruli and then carried down with the urine through the pelvis. It is striking as mentioned above that the infiltration of the pelvic wall was always most marked beneath the lining of the papilla. The eosinophilic leucocytes, because of their definite staining qualities, could be recognized in large numbers in these exudates. In order to compare the series of intravenous injections with those of intravesical injections, all the sections of the entire series were gone over once more to study in particular the question of infiltration about the pelvis, especially in its parietal portion, where it joined the ureter. Of the 13 cases of pyelitis there were two instances, animals 42 and 64, showing definite infiltration about the pelvis and extending down toward the

ureter. In animal 81 there was considerable infiltration limited rather strictly to the parietal wall of the pelvis and not extending down toward the ureter. In animal 75 there was considerable parietal infiltration of more chronic nature however. It is thus seen that in only a small proportion of cases was the parietal infiltration of any significance. The majority of the cases in the sections studied showed practically no infiltration.

In none of these experiments was any definite enlargement of the pelvic cavity to be made out. The pelvis, frequently distended with pus, never showed a pyonephrosis as did the original rabbit (R17.11).

The localization of colon bacilli in the kidney was very striking, 20 out of 31 animals showing renal lesions. Of equal importance is the fact that in only four instances were there any definite lesions outside the urinary tract which could be ascribed to the colon bacillus. These lesions consisted of one instance each of abscess of the seminal vesicle, diphtheritic colitis, hemorrhage of the caecum and generalized peritonitis. In our previous series, on the other hand, only 8 out of 66 animals showed kidney lesions and 21 of these 66 showed lesions elsewhere in the body. If the results of these two series of experiments are compared it is evident with what specificity this organism tends to localize in the kidney. In the present series of experiments coccidiosis was exceptional rather than the rule, while in the previous series it was quite prevalent. Whether or not this had anything to do with the localization of the colon bacilli in the other organs it is rather difficult to say.

The following is a detailed study of 4 rabbits from the time of intravenous injection of bacterial suspension to autopsy:

R17.19. Weight 1 pound 4½ ounces

February 23. Urine examined before injection: Microscopically there are no cells or casts; no albumin. Culture of urine shows no organism present in 1 cc. of urine. 1 cc. of thirty-six hour culture of urine of R17.11 was injected intravenously.

February 24. Urine examined. Microscopic examination negative; acid reaction; no albumin present.

February 26. Urine examined. About 10 pus cells to low power field. Temperature 40.7°.

February 27. Urine examined. About 10 pus cells to low power field. No albumin.

February 27, p.m. Reinjected as above with 1 cc. of twenty-four hour culture from urine of R17.11.

February 28. Urine examined. About 10 pus cells to low power field; acid reaction; albumin present.

March 1. Urine examined. Microscopic examination is negative.

March 2. Urine examined. About 10 pus cells to low power field; acid reaction; albumin present.

March 6. Urine examined. About 40 to 50 pus cells in low power field; acid.

March 7. Urine examined. 150 to 200 pus cells, single and in groups, are seen; no casts. The temperature was 39.8°.

March 8. 200 pus cells single and in groups; no casts.

March 9. Urine is loaded with pus cells and casts. Smear shows myriads of Gram-negative bacilli, which is the only organism present.

March 10. Catheterized. Urine loaded with pus cells Urine plated on plain agar and in broth. Plate is covered with colonies. Broth contains pure culture of Gram-negative bacilli. Plates contain only colonies of Gram-negative bacilli.

March 12. Diarrhea.

March 14. About 100 pus cells to low power field.

March 16. Many pus cells, single and in groups.

March 20. 150 to 200 pus cells in low power field.

March 26. Many pus cells, single and in groups; 5 to 6 granular and hyaline casts; albumin present.

March 29. Died.

Autopsy. Upon opening the peritoneal cavity the bladder contains a small amount of urine in which are found a few pus cells. The kidneys appear normal externally and on section. Both pelves are empty. There is a slight injection of the urethra. Culture of the bladder urine yields a Gram-negative bacillus. The other organs show nothing unusual.

Microscopic examination. Left kidney: There is considerable cloudy swelling of the epithelium of the convoluted tubules. There is a slight excess of eosinophil cells in the glomeruli. The cortex is otherwise negative. The medulla shows nothing unusual. The pelvis contains no exudate and the mucous membrane is not infiltrated with

pus cells. Right kidney: This resembles the left except that there is more marked infiltration of the glomeruli with eosinophil cells. There are no areas of infiltration but the eosinophil cells are scattered quite diffusely throughout the cortex. The medulla shows nothing unusual. The pelvis contains a purulent exudate consisting largely of polymorphonuclear leucocytes and some detritus. The mucous membrane throughout almost its entire extent is infiltrated with leucocytes,

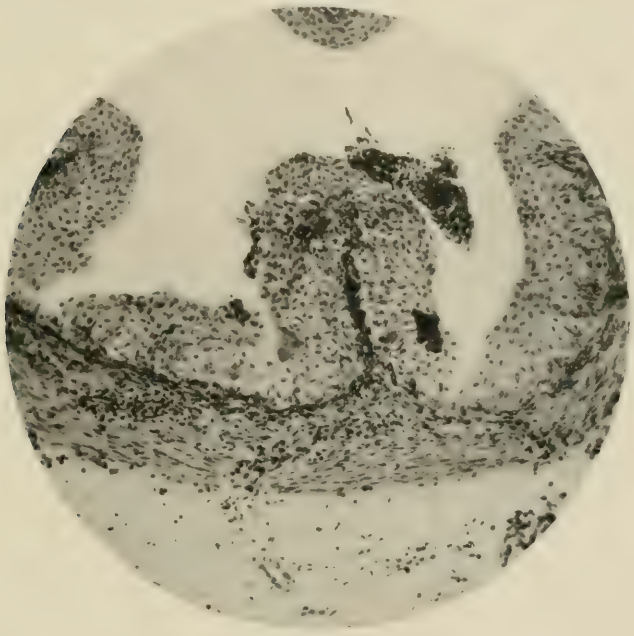

FIG. 2. PUS IN LUMEN; LEUCOCYTES IN TISSUE

many of them of the eosinophil type. The bladder shows nothing abnormal. The ureter shows slight desquamation of the epithelium in the lumen. There are no pus cells and no periureteral infiltration. The urethra shows nothing abnormal.

R17.27. Weigh 2 pounds 6 ounces

March 8. Urine: Microscopic examination negative; faintly alkaline; albumin absent. Injected intravenously with $1\frac{1}{4}$ cc. of a suspension

in 4 cc. of salt solution of 15 cc broth culture of B. coli from right pelvis of R17.11.

March 9. Urine: Microscopic examination negative.

March 10. Urine is loaded with pus cells.

March 12. Urine is loaded with pus cells. Trace of albumin is present.

March 14 to March 16. Many pus cells in urine.

Sacrificed.

Autopsy. Upon opening the peritoneal cavity the bladder vessels are distended and the tissues about the bladder somewhat edematous. The bladder is empty. The liver and gastro-intestinal canal are negative. The kidneys are of a dark brown red color and scattered irregularly throughout the surface are numerous opaque yellow areas varying in diameter from 1 to 3 mm. The ureters are definitely injected on the serous surface. From the cut section of both ureters pus can be expressed. Upon section of the kidney the cortex looks practically normal. Extending down from the cortex almost to the papillae are numerous yellow lines, widest at the cortex and narrowing toward the papillae. The pelves appear smooth, slightly injected. The right kidney pelvis contains pus. The lungs and heart show nothing unusual. The bladder shows numerous small hemorrhages on the surface. Cultures from the abscesses of the right and left kidney all show Gram-negative bacilli.

Microscopic examination. The bladder in the section studied shows practically an intact mucous membrane which in most areas is definitely infiltrated with polymorphonuclear cells. In some areas, just beneath the mucosa are seen small hemorrhages. In some areas in the same location there are collections of large number of leucocytes. The left kidney cortex shows considerable degeneration of the epithelium. In certain areas there is marked infiltration with polymorphonuclear leucocytes, distending the tubules and with considerable infiltration about them. In other areas the structure of the kidney is entirely lost and is just one large mass of leucocytes. This same holds true of areas in the medulla, extending down toward the papilla. The medulla is otherwise negative. The pelvis is filled with a purulent exudate. The lining of the pelvis does not show very much infiltration with leucocytes in the section studied. Section through the right kidney shows no abscesses in the cortex and only small areas of infiltration into the medulla. The pelvis is empty and there is no infiltration of the epithelium of the pelvis. The ureter shows nothing abnormal.

R17.36

March 17. Urine: Microscopic examination negative; albumin absent; faintly alkaline. Injected intravenously with 1 cc. of a suspension in 5 cc. of salt solution of 15 cc. of a broth culture (forty-eight hours) of B. coli from the pelvis of the right kidney of R17.26.

March 19. Urine shows about 20 pus cells in low power field.

March 20. Urine shows many pus cells, single and in groups; albumin absent; acid reaction.

March 21. Urine loaded with pus cells.

Died.

Autopsy done immediately after death. The bladder is distended with urine loaded with pus cells. The ureters are distended, especially so near the pelvis Pus is expelled from the cut end of both ureters. Right kidney: Externally there are seen many small abscesses on the surface. On longitudinal section pus exudes from the pelvis. The kidney otherwise appears normal. Left kidney: There are a few abscesses on the surface which is otherwise smooth. On section pus exudes from the pelvis. The spermatic sac is somewhat injected and contains some pus. The gastro-intestinal canal is entirely normal. There is coccidiosis of the liver and gall bladder and also of the heart. The lungs show slight congestion. Cultures of the bladder, right and left kidney pelves all show Gram negative-bacilli.

Microscopic examination. The lungs show marked congestion and in some small areas an inflammatory exudate. The bladder shows nothing abnormal. The kidney cortex shows marked swelling of the epithelium but no excess of cells in the glomeruli. In one area there is a purulent infiltration of several glomeruli and tubules extending down into the medulla. This infiltration disappears in the medulla in the section studied. The very tip of the papilla shows immediately underneath the mucous membrane a wide zone of infiltration with leucocytes. This zone gradually shades off into a single layer of cells underneath the lining of the pelvis. The pelvic mucosa shows considerable infiltration with polymorphonuclear leucocytes. The pelvis contains a small amount of pus. In one area there is a hemorrhage just beneath the pelvic mucosa. The lining of the upper portion of the ureter shows no infiltration nor is there any periureteral infiltration.

R17.81

April 9. Urine: Microscopic examination negative; albumin absent; acid reaction. Injected intravenously with 1 cc. of a suspension

in 3 cc. salt solution of 10 cc. broth culture of the organism from R17.64.

April 13. Urine: Microscopic examination negative.

April 16. Urine shows 30 to 40 pus cells and several casts.

April 19. Urine: Microscopic examination negative.

April 23. Urine: Microscopic examination negative.

April 25. Urine shows granular casts and many pus cells in clumps.

April 28. Urine shows 10 to 15 granular casts and a few small groups of pus cells.

May 1. Died.

Autopsy. The bladder urine contains casts and epithelial cells but no pus cells. The right kidney appears normal externally and on section. There is no pus in the pelvis of the right kidney. The left kidney resembles the right externally. On section there is a considerable amount of pus in the left pelvis. The stomach and intestinal canal are entirely normal. There is some coccidiosis of the gall bladder and liver. The autopsy is otherwise negative. Cultures from the bladder urine contain Gram-positive cocci and Gram-negative bacilli. Culture from the left kidney pelvis shows only Gram-negative bacilli.

Microscopic examination. Left kidney: The cortex shows inflammatory infiltration. The glomeruli appear normal and the medulla shows no definite areas of acute infiltration. The pelvis contains considerable amount of purulent exudate but only slight infiltration of the wall over the papillae. The submucous infiltration is more marked in the parietal portion of the pelvis. The right kidney shows a normal cortex and medulla. There is no pus in the pelvis and no evidence of infiltration of the wall.

INTRAVESICAL INJECTIONS

A total of 15 animals received intravesical injections of from 5 to 8 cc. of a broth culture of Bacillus coli communior. At the time of the injection the bladder was first drained of its urine and this urine was cultured in each instance. In no instance was there a Gram-negative bacillus found and in none were there more than a few organisms per cubic centimeter of urine. The broth culture was allowed to run into the bladder by gravity. In order to make sure that by this procedure no material reached the ureters, we injected 3 animals with gentian violet in the

TABLE 2

Intravesical injections

ANIMAL	MICRO-ORGANISMS FROM	DATE OF INJECTION	DATE OF DEATH	KIDNEY			CYSTITIS	URINE		REMARKS
				Pyelitis	Abscess	B. coli		Pus	B. coli	
R31	R26	March 17	April 17	0	0	0	∗	∗	∗	
R32	R26	March 17	April 9	0	0	0	∗	∗	∗	
R47	R36	March 22	April 6	Bilateral	0	∗	∗	∗		Heart culture negative
R49	R36	March 22	March 31	Bilateral	0	∗	0	∗	∗	Heart culture negative
R50	R42	March 29	April 11	0	0	L ∗ R 0	∗	∗	∗	
R51	R42	March 29	April 11	Bilateral	0	∗	∗	∗	∗	Pus in pelves, ureters distended
R52	R42	March 29	April 11	Bilateral	0	L 0 R ∗	∗	∗	∗	Entirely normal kidney macroscopically
R53	R42	March 29	April 11	0	0	0	∗	∗	∗	
R54	R42	March 29	April 11	0	0	L ∗ R 0	∗	∗	∗	
R66	R41	March 31	April 11	Bilateral		R ∗	∗	∗	∗	
R68	R41	March 31	May 4	Unilateral	0	R ∗ L 0	0	∗	∗	
R77	R64	April 9	May 5	Bilateral	0	—	—	∗	∗	
R78	R64	April 9	April 16	Bilateral	0	—	∗	∗	∗	Bladder culture. Mixed B. coli and staphylococcus
R85	R47	April 9	April 21	Bilateral	0		0	∗		
R86	R47	April 9	May 9	Unilateral	0	R ∗ L ∗		∗	∗	Pus in vagina
Total, 15				10	1	2				

same manner. In none of them were we able to find the bladder markedly distended or any evidences that the fluid had passed into the ureters. Since these experiments we have used this same technic with another series of rabbits and found much to our disappointment that the infection was limited to the bladder in the great majority of instances.

Of this first series of 15 animals, 10 developed pyelitis, 8 bilateral and 2 unilateral. In one of the instances of unilateral pyelitis the culture from both kidneys was positive but evidence of inflammation was present in only one kidney. In one case only, R66, were there cortical abscesses. These abscesses were present in a small group in only one area of each kidney. They differed in this respect from the abscesses following intravenous injection which were always diffusely scattered over the surface. Furthermore they were surrounded by quite a wide zone of hemorrhagic infiltration.

In two instances, R49 and R54, there were no findings either macroscopic or microscopic but we were able to cultivate the Bacillus coli communior from the kidney pelvis. All cultures taken from the heart's blood at autopsy were found to be sterile.

If we compare the pathology of the intravesical injections with that of the intravenous injections, we find that the parietal infiltration is much more evident in the former. Although some of the intravesical injections caused infiltration of the mucous membrane of the papilla, it is found only in those cases in which the infiltration of the parietal membrane is very marked. In some instances there is parietal infiltration but absence of any submucous infiltration of the papilla. In rabbit 52 there is definite parietal infiltration with slight infiltration of the papilla. Animal 66 of all this series probably best shows the course of the infection. The bladder shows very marked infiltration of the wall. The section of the ureter entering the bladder shows a definite polymorphonuclear infiltration, both in the muscle layers as well as in the periureteral tissue. Near the uretero-pelvic junction, the ureteral wall is densely infiltrated with an acute exudate and the parietal portion of the pelvic mucosa is densely infiltrated with cells. The abscesses, as men-

tioned above, were limited in each kidney to a small portion of the cortex and the direct connection between the abscesses and the pus-filled tubules down to the pelvic cavity could be demonstrated. The sections of the bladder, ureter and kidney (figs. 2 and 3) especially when these are compared with sections of the same organs from animals receiving intravenous injections, readily show the course of the infection and this can be traced from the bladder to the cortex of the kidney. From this severest type of ascending infection, we have all transitions to the type in which we were able to find only the colon bacillus in culture from the pelvis of the kidney without any lesions, macroscopically or microscopically. At this point we do not wish to go more particularly into the question of the ascending infection, except to say that it seems to us that these experiments, by the mode of injection and by the pathological condition found, prove quite definitely that the infection traveled up the periureteral lymphatics to the kidney, rather than that from a primary cystitis a secondary infection of the kidney occurred by way of the blood stream. We hope at a later date to go more minutely into the histological findings in cases of ascending infection. Further experiments in this connection are at present under way.

The following is a detailed study of 4 rabbits receiving intravesical injections of bacterial suspensions.

R17.54

March 29. Urine: Microscopic examination negative; albumin absent; acid reaction. Catheterized specimen of urine was plated; no growth. 5 cc. broth forty-eight hour culture from the pelvis of the left kidney and bladder of R17.42 were injected intravesically.

March 30. Urine shows 8 to 10 pus cells.

April 2. Urine shows many pus cells and 6 to 8 granular and hyaline casts.

April 4. Urine shows 20 to 30 single cells and several groups.

April 10. Urine loaded with pus cells and several casts.

April 11. Sacrificed.

Autopsy. The bladder is somewhat edematous and is filled with urine containing many pus cells mostly in troups. The ureters and kidneys show nothing abnormal, externally or on section. Cultures

from the heart and the right kidney are negative; from bladder and left kidney positive for Gram-negative bacilli.

Microscopic examination. The bladder: The epithelium is markedly infiltrated with pus cells. The subepithelial layer and also deeper layers of the bladder show considerable polymorphonuclear infiltration. Right kidney: The cortex and medulla show no evidence of infiltration. The pelvis is empty and the mucosa shows no infiltration. Section

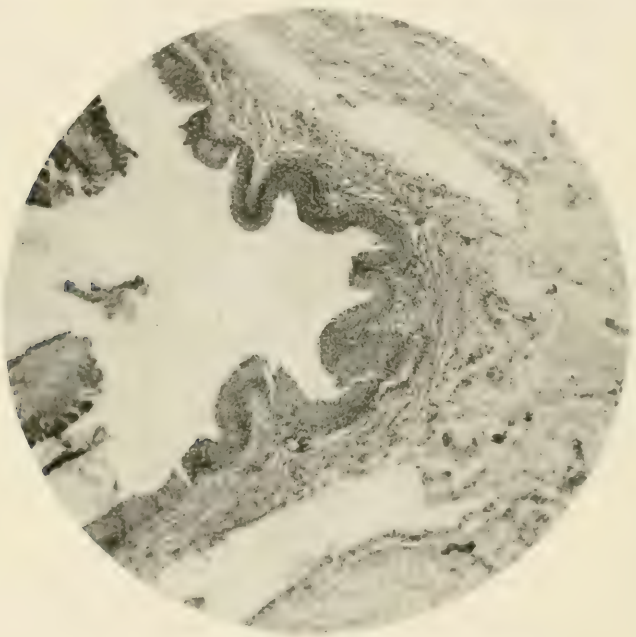

Fig. 3. Pyelitis; Peri-Pelvic Inflammation

of the ureter shows nothing abnormal. Left kidney: The cortex and medulla show nothing abnormal. There is no exudate in the pelvis. The epithelium of the pelvis shows no infiltration at any point. The section of the ureter shows an empty lumen and no infiltration of the mucosa or of the periureteral tissues.

R17.66

March 31. Urine: Microscopic examination shows some epithelial cells; acid reaction; albumin absent. Catheterized specimen plated: One colony on plate—staphylococcus albus.

March 31. 6 cc. broth twenty-four hour culture of organisms from R17.41 were injected intravesically.

April 2. Urine shows many pus cells, single and in groups.

April 4. Urine is loaded with pus cells, single and in groups; no casts.

April 10. Urine loaded with pus cells and casts. Animal in poor condition.

April 11. Sacrificed.

Autopsy. Upon opening the peritoneum the bladder contains a small amount of urine filled with pus cells and casts. The bladder wall appears slightly edematous. The kidneys externally show a group of small hemorrhagic lesions with yellowish centers. On section these lesions are seen to extend through the cortex down into the medulla. There too they appear rather hemorrhagic (see figure 3). The pelves are distended with pus. Cultures from the bladder and right kidney show pure culture Gram-negative bacilli. Culture of the heart's blood is negative.

Microscopic examination. Right kidney: Sections taken from three serial blocks.

Block 1. For the most part the kidney cortex appears entirely normal. In one area, however, there are numerous small infiltrations of polymorphonuclear leucocytes near the glomeruli and surrounding tubules. In the central one of these areas the process has spread breaking through the tubules and showing quite a wide zone of infiltration. Extending from this area there are several streaks running down into the medulla and on cross section small groups of tubules in the medulla are seen filled with a purulent exudate, the walls of which show considerable polymorphonuclear infiltration. The pelvis contains pus. The walls in some areas show polymorphonuclear infiltration which, however, is not very marked.

Block II. In the section from this block the cortex appears entirely normal with the exception of infiltration in one glomerulus. The upper portion of the medulla also appears perfectly normal and no streaks are seen running down. In the lower portion of the medulla cut in transverse section are seen numerous groups of tubules densely filled with purulent exudate. Many of them are surrounded by a collar of infiltration. In this section the pelvis contains pus and shows very much more marked infiltration of the pelvic mucosa.

Block III. This runs through the tip of the papilla and shows a large opening of the tubules filled with a purulent exudate and showing

in numerous places dense infiltration in the peritubular tissue. The epithelium on the side of the papilla seems to be thickened and on sections shows small groups of pus cells surrounded completely by epithelium. The mucosa of the pelvis shows some infiltration, but the subepithelial infiltration is much more marked. The cortex in this section shows nothing abnormal. The loose areolar tissue about the pelvis shows a large excess of polymorphonuclear cells. The vessels show nothing unusual.

R17.68

March 31. Urine: Microscopic examination negative; albumin absent; alkaline reaction. Catheterized specimen plated: one colony staphylococcus albus in 1 cc. of urine. Six cubic centimeters of a twenty-four hour broth culture of organisms from R17.41 were injected into the bladder.

April 2. Urine shows many pus cells, single and in groups.

April 4. Urine shows 50 to 60 pus cells in low power field but no casts.

April 10. Urine shows 4 to 5 pus cells but no casts and no débris.

April 13. Urine shows 40 to 50 pus cells.

April 16. Urine loaded with pus cells.

April 19. Urine shows strings of pus but few in the remainder of the urine.

April 23. Impossible to obtain specimen.

April 25. Urine loaded with pus cells and strings of pus, also 8 to 10 granular casts; albumin present.

April 28. Urine shows 18 to 20 granular and hyaline casts and many pus cells, also some strings of pus.

May 2. Urine loaded with pus cells and casts.

May 4. Dead.

Autopsy. Upon opening the peritoneal cavity the bladder urine contains many pus cells and casts. The bladder is normal. The vagina contains a small amount of pus. The ureters are somewhat injected, particularly the right. The kidneys appear normal externally and on section, except for pus in the pelvis of the right. The left pelvis was empty. The other organs are entirely negative. The bladder and right kidney pelvis yield in culture only Gram-negative bacilli. The culture from the left kidney pelvis was negative.

Microscopic examination. The right kidney shows a normal cortex except in one area there is some lymphoid infiltration about the ves-

sels extending down between several of the tubules. In the medulla there are several areas where there is infiltration with lymphocytes. Near the tip of the papilla several tubules are filled with an exudate containing polymorphonuclear cells. The pelvis is practically empty. The pelvic mucosa shows in some areas slight submucous infiltration. Left kidney: This shows no changes in the cortex, medulla, or pelvis.

R17.86

April 9. Urine: Microscopic examination negative; albumin absent; acid reaction. Five cubic centimeters of a broth culture of R17.47 were injected intravenously. Catheterized specimen of urine plated.

April 11. First specimen plated; shows 3 colonies of Gram-positive coccus.

April 13. Urine loaded with casts; granular.

April 15. Urine loaded with pus cells, and 20 to 50 granular casts.

April 19. Urine shows 10 to 12 pus cells.

April 23. Urine shows many pus cells and 5 to 6 granular casts.

April 28. Urine shows 20 to 30 pus cells and 2 to 3 granular casts, also much débris.

May 2. Urine loaded with pus cells and contains many casts. Urethra swollen and apparently infected.

May 9. Animal died.

Autopsy done immediately. The bladder is distended with urine which contains many pus cells and casts. The ureters and bladder are normal externally. Both kidneys appear normal externally. The left pelvis contains pus on section. The right pelvis contains none. There is some pus in the vagina. The autopsy is otherwise negative. Culture from both kidneys and bladder yield pure culture Gram-negative bacilli.

Microscopic examination. The right kidney cortex and medulla show no evidence of any acute inflammatory infiltration. There are few collections of mononuclear cells in the cortex. The pelvis is empty and there is no apparent infiltration of the wall of the pelvis. The left kidney also shows an area of lymphoid infiltration in the cortex. The medulla is negative. There is a purulent exudate in the pelvis but practically no infiltration of the pelvic mucosa. The ureters show nothing abnormal.

TABLE 3

ANIMAL	MICRO-ORGANISMS FROM	DATE OF INJECTION	DATE OF DEATH	KIDNEY			CYSTITIS	URINE		REMARKS
				Pyelitis	Abscess	B. coli		Pus	B. coli	
Oral injections										
R55	R42	March 29 March 31 April 3	April 22	0	0	R ✱	0	0	✱	Autopsy several hours after death
R56	R42	March 29 March 31	April 3	0	0	0	0	0	0	Urine contains casts
R57	R42	March 29 March 31 April 3	April 27	0	0		0	0	✱	Mixed bladder culture. B. coli and Gram-positive cocci. Granular and hyaline casts in urine
R58	R42	April 3	May 8	0	0	0	0	0	0	
R59	R42	April 3	April 4	0	0	0	0	0		
R69	R41	March 31 April 5	April 26	0	0	0	0	0	0	Casts in urine, no pus
R70	R41	April 5	April 16	0	0	R0	0	0	0	
R71	R41	April 5	April 5	0	0	0	0	0	✱	
R72	R41	April 5	April 7	0	0	0	0			
Rectal injections										
R79	R64	April 10	April 30	0	0		0			Casts in urine
R80	R64	April 10	April 16	0	0	0	0	0	0	
R87	R47	April 10	April 27	0	0		0	0	✱	Casts in urine
R88	R47	April 10	April 21	0	0		0			Injection of intestines

0 = absent
✱ = present

ORAL INFECTIONS

As a control, we fed 9 animals with the strain of Bacillus coli communior which we employed in these experiments. Most of the animals received 8 cc. of the culture on three occasions A number of the animals showed a marked degree of diarrhea but in no instance did any infection develop in the urinary tract. In one instance in which the autopsy was done some hours after death, a colon bacillus was isolated from the kidney and bladder. In two other cases in which the autopsy was done shortly after death, the Bacillus coli was obtained from the bladder urine. Eight of the nine animals apparently died from the results of the feeding but the urinary tract was free from any involvement.

R17.56

March 29. Urine contains a few epithelial cells; no albumin and acid reaction. Fed 5 cc. forty-eight hour broth culture from the pelvis of the left kidney and bladder of R17.42.

March 30 and March 31. Urine: Microscopic examination is negative.

March 31. Fed 8 cc. forty-eight hour broth of R17.42.

April 2. Urine shows a large number of granular casts, about 20 to 30. No pus cells.

April 3. Dragging left hind leg and so sacrificed.

Autopsy. The bladder is markedly distended with urine containing a large number of casts but no pus cells. The other organs are entirely negative. In the muscles of the right thigh are numerous and extensive hemorrhages. Cultures of the muscle, the bladder urine and the pelves of the right and the left kidneys are negative.

Microscopic examination. The cortex of both kidneys shows nothing abnormal. The medulla contains numerous epithelial casts in the collecting tubules. The pelves are empty and the pelvic lining shows nothing abnormal.

R17.79

April 9. Urine: Microscopic examination negative; albumin absent acid reaction.

April 10. Injected the rectum with 8 cc. of a twenty-four hour broth culture of organisms from R17.64.

April 13. Urine: Examination is negative.
April 16. Urine loaded with casts and 20 to 30 pus cells.
April 19. Urine shows 20 granular casts but no pus cells.
April 23. Urine: Microscopic examination is negative.
April 25. Urine shows 30 to 40 casts and 10 to 15 pus cells.
April 28. Urine: Microscopic examination is negative.
April 30. Found dead.

Autopsy. Upon opening the peritoneal cavity the bladder is distended with urine which contains no pus cells or casts. The kidneys and ureters are negative. The other organs appear entirely normal.

Microscopic examination. The cortex, medulla and pelvis of both kidneys are entirely normal.

RECTAL INJECTIONS

In order to determine the effect of rectal injection of this same organism, we injected 4 animals with 8 cc. of broth culture. Several of the animals showed considerable congestion of the lower portion of the intestinal canal. The kidney and bladder findings were entirely negative however. All 4 of these animals died, apparently as a result of the injections.

SUMMARY

Pyelitis has been experimentally produced in the rabbit by the intravenous injection and by the intravesical injection of:

Bacillus coli communior isolated from spontaneous pyelitis in the rabbit.

Pyelitis was produced in 17 of 31 rabbits injected into the ear vein. Ten per cent of the rabbits had also abscesses in the kidney. Three rabbits had abscesses without a pyelitis.

Pyelitis was produced in 10 out of 15 rabbits by intravesical injection. In only one instance were there abscesses in the kidney.

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THE BACTERIOLOGY OF THE URINE IN CHILDREN WITH GASTROINTESTINAL DISEASES *

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Many observers have noted that the occurrence of cystitis in infancy occasionally correlates the incidence of gastrointestinal diseases. This observation was interpreted as lending support to any of the 3 commonly offered theories regarding the route of infection of this disease. Those believing in the lymphogenous or hematogenous routes of infection assumed that the injured intestinal mucosa in gastrointestinal diseases facilitated the passage of colon bacilli into the bladder either directly or by way of the blood stream. Those advocating the ascending route of infection regarded the occurrence of frequent stools as likely sources of urethral contamination with resulting cystitis. This idea gained additional support on account of the apparent predominance of cystitis in female infants. There have, however, been very few studies establishing the actual conditions of the urinary tract during gastrointestinal diseases.

Trumpp[1] studied the bacteriology of the urine in 16 infants with "enteritis follicularis." 3 gave negative results. 13 showed a bacilluria, 9 of which were in girl babies. Williams[2] cultured the catheterized specimens of a series of adult patients to determine the difference between specimens obtained from individuals with enteric diseases and from those without such diseases. However, the character of her cases and the methods of study are so incompletely explained as to preclude any conclusions. It therefore seemed important to study the bacteriology of the urine in gastrointestinal diseases, not only to determine the frequency of bacterial contamination as compared with the urine of normal infants, but to ascertain the relative frequency of such infection in male and female infants.

The patients studied were admitted to the Children's Hospital during the summer of 1916, during which time a terrific heat spell prevailed in Chicago. They represented various types of all the known forms of gastrointestinal diseases and were generally

* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital, Chicago.

of a very severe nature as exhibited by the extremely high mortality rate. With a very few exceptions these patients were infants under 2 years of age.

In this investigation, the methods used were those described in previous studies from this laboratory[3],[4]. The male babies were catheterized with a silk elastic catheter which was sterilized by immersion in 1% lysol solution. To ascertain the accuracy of the method, a few controls were made by washing sterile water through the catheter, and inoculating the washings into media similar to those used in the study of the urine specimens. The results of 6 controls were as follows: 2 remained sterile, 2 grew a single colony of bacillus subtilis, 1 grew 2 colonies of the same organism and 1 grew 6 colonies of staphylococci.

63 separate specimens were studied. Of these, in 42 instances cultures were made from 2 separate portions of the urine. The first specimen approximately represents the flora of the urethra and bladder, the second specimen that of the bladder alone. In the remaining 21 instances, the amount of urine obtained was insufficient to make this division and the entire specimen was inoculated. The respective groups are given in separate tables. Besides the bacteriological examination, the tables indicate the chemical analyses of the urine, the highest point of the body temperature on the day of catheterization, the duration of the illness until the day of catheterization, the diagnosis and outcome of the disease.

Table 1 gives the results of catheterizations in 14 girls, from whom only 1 specimen was cultured. In these 14 instances, 10 showed innumerable colonies (expressed in the table by the + + sign). Of these 10 bacteriurias, in 8 instances the offending organism was a gram-negative bacillus, in 2 instances a staphylococcus. In the remaining 4 instances, 2 showed gram-negative bacilli; 1, staphylococci; and 1, a diphtheroid bacillus. The number of organisms in these 4 was as follows: In 2 instances, 1 or less than 1 colony per cubic centimeter; in 1 instance, 6 colonies per cubic centimeter; in 1 instance 10-15 colonies per cubic centimeter.

Table 2 gives the results of catheterizations in 7 boys from whom only 1 specimen of urine was cultured. The staphylococcus was the prevailing organism. In no instance was a Gram-negative bacillus encountered. In no instance was there an actual

bacteriuria, the colonies occurring in such small numbers that they could easily be considered contaminations from the urethra.

Table 3 gives the results of catheterizations in 27 girl patients from whom 2 specimens were obtained. The bacteriology of specimen 2 in these cases was as follows: In 7 instances there were innumerable colonies (Gram-negative bacilli); in 14 instances there were from 6-40 Gram-negative bacilli per cubic centimeter of urine either alone or in combination with other organisms; in 4 instances there were from 3-30 organisms, other than Gram-negative bacilli per cubic centimeter; 1 specimen remained sterile and one gave a growth of less than 1 colony per cubic centimeter (B. subtilis). Specimen 1 in the series, as would be expected, shows a greater number of organisms per cubic centimeter.

Table 4 gives the results of catheterizations of 15 boy patients from whom 2 specimens were obtained. In only 1 instance was there a bacilluria, in 1 other instance about 50 colonies of Gram-negative bacilli per cubic centimeter of urine, in 4 instances less than 5 colonies of Gram-negative bacilli, per cubic centimeter of urine, either alone or in combination with other organisms, while in 9 instances no Gram-negative bacilli were encountered in the second portion of urine. In this series, the bacteriology of the first specimen was as follows: 3 instances showed innumerable bacteria, in 2 of which the Gram-negative bacillus was present either alone or in combination with other organisms. In 2 instances there were over 20 colonies of Gram-negative bacilli per cubic centimeter of urine, in 9 instances less than 10 colonies per cubic centimeter of urine. In none of the latter 9 was there a Gram-negative bacillus present, the prevailing organisms being Gram-positive cocci and diphtheroid bacilli, the same micro-organisms which have been reported as occurring in the urethra of normal children[3].

DISCUSSION

Combining the totals of Tables 1 and 3, which are the 2 series of girl babies, we find 32 instances in a total of 41 catheterizations which show the presence of Gram-negative bacilli in Specimen 1. Considering only second specimens, we find 21 instances in a total of 27 which present similar findings. The difference is negligible.

TABLE 1—CATHETERIZATIONS—GIRL PATIENTS FROM WHOM ONLY 1 SPECIMEN WAS CULTURED.

NAME	AGE	URINALYSIS	COLONIES PER C.C.	TYPES OF ORGANISMS	TEMP.	DURATION OF DISEASE	DIAGNOSIS AND REMARKS.
C. K.	9 mo.	Albumin, faint trace. No casts	1	Gram. pos. diphtheroid bacillus.	100.	10 days	Ileocolitis. Recovered.
L. M.	14 mo.	.....	6	Gram neg. bacilli.	100.	1 day	Gastroenteritis. Recovered.
L. R.	19 mo.	Albumin, faint trace. Few hyaline casts. Very few W. B. C.	++	Gram neg. bacilli.	104.	8 days	Enteritis pneumonia. Died.
R. C.	9 mo.	Albumin, neg.	++	Gram neg. bacilli.	98.4	12 days	Enteritis. Recovered.
V. R.	2 mo.	Negative.	++	Gram neg. bacilli.	98.2	14 days	Gastroenteritis. Recovered.
S. G.	6 mo.	Albumin, pos. Numerous W. B. C.	++	Gram neg. bacilli.	102.2	18 days	"Intoxication." Died.
S. J.	4 mo.	Albumin, pos. Casts pos.	++	Gram neg. bacilli.	104.8	3 days	"Intoxication." Died.
G. P.	2 mo.	Negative.	++	Staphylococci.	99.	7 days	Enteritis. Died.
M. G.	7 mo.	Albumin, neg. 10-20 W. B. C.	++	Gram neg. bacilli.	103.	7 days	Enteritis. Sent home.
F. O.	2 mo.	Negative	10-15	Staphylococci.	97.6	2 weeks	Enteritis atrophy. Died.
M. S.	10 mo.	Albumin, pos. W. B. C. numerous.	++	Gram neg. bacilli.	100.	10 days	Enteritis cystitis. Recovered.
S. H.	2 mo.	Albumin, pos.	++	Gram neg. bacilli.	99.2	8 days	Gastroenteritis. Recovered.
B. M.	5 years	Negative	1	Gram neg. bacilli.	100.	9 days	Gastroenteritis. Recovered.
I. T.	6 mo.	Negative	++	Staphylococci.	101.4	7 days	Enteritis. Recovered.

TABLE 2—CATHETERIZATIONS—BOY PATIENTS FROM WHOM ONLY 1 SPECIMEN WAS CULTURED.

NAME	AGE	URINALYSIS	COLONIES PER C.C.	TYPES OF ORGANISMS	TEMP.	DURATION OF ILLNESS	DIAGNOSIS AND REMARKS.
J. H.	17 mo.	Albumin, neg. Few W. B. C.	1	Staphylococci.	99.5	7 days	Enteritis. Recovered.
J. C.	2 yr.	Albumin, pos. Microscopic neg.	25	Staphylococci.	101.	10 days	"Intoxication." Died.
J. S.	2 yr.	Negative.	12	Staphylococci.	99.	5 days	Ileocolitis. Recovered.
R. D.	15 mo.	Albumin, faint trace. 1-3 W. B. C. to H. P. F.	4	Staphylococci Gram pos. diplococci.	99.6	7 days	Gastroenteritis. Recovered.
V. D.	7 mo.	Negative.	30	Staphylococci.	101.8	6 weeks	Enteritis. Recovered.
J. S.	13 mo.	Albumin, pos. Microscopic neg.	10	Staphylococci.	103.	7 days	Enteritis. Recovered.
M. W.	5 mo.	.....	1	Staphylococci.	99.	2 weeks	Enteritis. Died.

TABLE 3.—CATHETERIZATIONS.—GIRL PATIENTS FROM WHOM 2 SPECIMENS WERE CULTURED.

NAME	AGE	URINALYSIS	CULTURES OF SPEC. 1.		CULTURES OF SPEC. 2.		DURATION OF ILLNESS	TEMP.	DIAGNOSIS AND REMARKS
			COLONIES PER C.C.	TYPES OF ORGANISMS	COLONIES PER C.C.	TYPES OF ORGANISMS			
A. L.	5 mo.	Albumin, pos. Hyaline and granular casts. Few W.B.C.	++	Streptococcus and B. Coli.	++	Streptococcus and B. Coli.	8 days	98.8	"Intoxication." Died.
B. M.	6 yr.	Albumin, faint trace. Few hyaline casts. Very few W.B.C.	22	Gram neg. bacilli.	14	Gram neg. bacilli.	24 hours	98.6	Ileocolitis. Improved.
O. M.	6 mo.	Albumin, faint trace. 8-10 W.B.C. to H.P.F.	++	Gram neg. bacilli.	15	Gram pos. diplococci Gram neg. bacilli.	5 days	97.4	Gastroenteritis. Recovered.
O. M.	6 mo.	Albumin, neg.	12	Gram neg. bacilli and diplobacilli.	12	Gram neg. bacilli.	9 days	98.	Gastroenteritis. Recovered.
E. K.	10 mo.	Albumin, pos. Numerous W.B.C.	++	Gram neg. bacilli.	++	Gram neg. bacilli.	18 days	102.8	Ileocolitis and pyelocystitis. Died.
M. S.	3 mo.	Albumin, neg. Microscopic, neg.	++	Gram neg. bacilli.	++	Gram neg. bacilli.	3 weeks	99.4	Gastroenteritis and Infantile atrophy. Died
G. E.	6 mo.	....	18	Gram neg. bacilli and staphylococci	6	Gram neg. bacilli.	2 days	104.	Lobar pneumonia and furunculosis. Died.
N. T.	15 mo.	Albumin, neg. Microscopic neg.	++	Gram neg. bacilli.	++	Gram neg. bacilli.	2 weeks	100.	Enteritis and furunculosis. Improved.
J. S.	5 yr.	Albumin, faint trace. Few W.B.C.	1	Gram neg. bacilli.	7	Gram neg. bacilli	3 days	101.	Gastroenteritis. Died.
M. N.	5 mo.	Albumin, neg. Microscopic neg.	10	Gram neg. bacilli and streptococci.	20	Gram neg. bacilli.	4 days	100.	Gastroenteritis. Improved.
J. T.	9 mo.	Albumin, neg. No W.B.C.	3	Staphylococci.	7	Gram neg. bacilli and staphylococci	3 days	99.4	Enteritis. Improved.
J. B.	9 mo.	Albumin, neg. Microscopic neg.	4	Gram pos. diphtheroid bacillus.	None	.....	8 days	102.2	Enteritis and otitis media.
E. G.	15 mo.	Albumin, neg. Microscopic neg.	10	Staphylococci.	13	Staphylococci.	12 days	100.2	Enteritis. Improved.
M. D.	12 mo.	Albumin, neg. Microscopic neg.	++	Gram neg. bacilli.	++	Gram neg. bacilli.	6 days	101.	Enteritis. Improved.
G. K.	9 mo.	Albumin, neg. Microscopic neg.	10	Streptococci and Gram neg. bacilli.	10	Streptococci and Gram neg. bacilli	1 month	99.	Rickets and enteritis. Improved.
F. T.	8 mo.	Albumin, faint trace. 2-5 W.B.C. to H.P.F.	40	Gram neg. bacilli.	10	Gram neg. bacilli.	10 days	101.8	Gastroenteritis and furunculosis. Died.
C. M.	9 mo.	Albumin, faint trace. Moderate No. W.B.C.	++	Gram neg. bacilli.	++	Gram neg. bacilli.	2 weeks	?	Enteritis. O.P.D.
L. S.	6 mo.	Albumin, neg.	21	Gram neg. bacilli.	?	Gram neg. bacilli and cocci.	?	99	Enteritis and infantile atrophy. Died.
S. G.	6 mo.	Albumin, pos. W.B.C., pos.	30-40	Gram neg. bacilli.	30-40	Gram neg. bacilli.	3 weeks	102.4	"Intoxication." Died.
V. K.	2 mo.	Albumin, pos. Moderate No. W.B.C.	2-3	Gram neg. bacilli.	3	Gram neg. diplococci.	2 weeks	98.6	Enteritis. Died.
S. B.	4 yr.	Albumin, neg. Microscopic neg.	10	Gram neg. bacilli.	15	Gram neg. bacilli.	5 days	102.2	Ileocolitis. Improved.
C. K.	1 yr.	No Albumin.	4	Gram neg. bacilli and staphylococci.	=1	B. Subtilis.	2 weeks	99.	Ileocolitis. Improved.
C. K.	1 yr.	No Albumin. Microscopic neg.	25	Gram neg. bacilli.	15	Gram neg. bacilli.	4 weeks	98.	Ileocolitis. Improved.
M. W.	14 mo.	Pos. Albumin. Pos. W.B.C.	++	Gram neg. bacilli.	++	Gram neg. bacilli.	10 days	100.8	Ileocolitis. Improved.
M. W.	14 mo.	Few casts. Very few W.B.C.	5-10	Staphylococci.	5-10	Staphylococci.	4 weeks	100.	Ileocolitis. Died.
A. P.	2 yr.	Albumin, neg. Microscopic neg.	4	Gram neg. bacilli and Gram pos. diplococci.	15	Gram neg. bacilli.	8 days	102.	Ileocolitis. Home against advice.
A. P.	2 yr.	Albumin, neg. Microscopic neg.	6	Cocci, Gram neg.	20-30	Cocci, Gram neg.	3 weeks	101.	Ileocolitis. Home against advice.

TABLE 4—CATHETERIZATIONS—BOY PATIENTS FROM WHOM 2 SPECIMENS WERE CULTURED.

NAME	AGE	URINALYSIS	CULTURES OF SPEC. 1.		CULTURES OF SPEC. 2.		DURATION OF ILLNESS	TEMP	DIAGNOSIS AND REMARKS
			COLONIES PER C.C.	TYPES OF ORGANISMS	COLONIES PER C.C.	TYPES OF ORGANISMS			
E. P.	9 mo.	Albumin, faint trace. Few W.B.C.	3	B. Subtilis. Diphtheroid bacillus.	3	B. Subtilis. Staphylococci.	2 weeks	98.	Enteritis. Recovered.
L. T.	9 mo.	Negative.	4	B. Subtilis. Gram pos. diplococcus.	3	Gram pos. diplococcus.	3 weeks	98.8	Enteritis and atrophy. Recovered.
L. T.	9 mo.	Albumin, pos. 3-4 W.B.C. to field.	++	Gram. neg. bacilli.	1	Staphylococci.	2 days	98.8	Enteritis and atrophy. Recovered.
J. K.	8 mo.	Albumin, faint trace. Microscopic neg.	1	B. Subtilis.	10	Staphylococci.	1 day	104.	Thermic fever. Died.
H. C.	3 weeks	Albumin, faint trace. Microscopic neg.	20 +	Gram. neg. bacilli	50 +	Gram. neg. bacilli.	12 days	98.8	Enteritis. Improved.
S. B.	6 weeks	Albumin, pos. 3 W.B.C. to H.P.F.	++	Gram. neg. bacilli.	++	Gram. neg. bacilli.	4 days	100.	Enteritis. Died.
H. S.	7 mo.	Negative.	20 +	Staphylococci and Gram neg. bacilli.	2 +	Staphylococci and Gram neg. bacilli.	2 weeks	98.4	Gastroenteritis. Improved.
E. R.	9 mo.	Albumin, trace. Microscopic, neg.	10	Staphylococci.	2	Staphylococci and Gram neg. bacilli.	10 days	99.4	Gastroenteritis. Improved.
E. R.	9 mo.	Albumin, neg. Microscopic, neg.	3	Staphylococci and B. Subtilis.	5	Staphylococci.	4 weeks	99.	"Intoxication." Died.
J. D.	6 mo.	Albumin, faint trace. Microscopic, neg.	5	B. Subtilis.	?	B. Subtilis and Gram neg. diplocilli.	2 weeks	99.	Gastroenteritis. Died.
G. C.	6 mo.	Negative.	2	Gram pos. bacillus and coccus.	3	Gram neg. bacilli	3 weeks	100.4	Dyspepsia. Improved.
E. H.	10 mo.	Albumin, faint trace. Microscopic, neg.	9	Staphylococci and Gram neg. bacilli.	4	Gram neg. bacilli	4 weeks	101.2	Gastroenteritis. Died.
H. J.	1 yr.	Negative.	1	Staphylococci.	2	Staphylococci and B. Subtilis.	5 weeks	99.2	Ileocolitis. Improved.
I. S.	1 yr.	Albumin, pos. Microscopic, neg.	0	.....	3	Gram pos. coccus.	12 days	101.	Enteritis. Improved.
E. M.	9 mo.	Negative.	++	Gram neg. bacilli, Gram pos. diplococci and diphtheroid bacilli.	12	Staphylococci.	12 days	98.2	Enteritis. Improved.

Combining the totals of Tables 2 and 4, which represent the 2 series of boy babies, we find 6 instances in a total of 22 catherizations which show the presence of Gram-negative bacilli either in specimen 1 or specimen 2 (see Table 5).

Helmholz and Beeler mention the frequent occurrence of pathologic constituents (albumin, white blood cells and casts) in the urine of patients with extra-urinary infections. In the present series, this circumstance is even more noticeable. While these instances are relatively more frequent in patients presenting a bacilluria, the relationship is by no means definite. The significance of such findings should be considered reservedly and does not always, just as Helmholz and Beeler emphasize, constitute the basis for a diagnosis of pyelocystitis. Catheterization must be resorted to more frequently in establishing such a diagnosis. If carefully done, this procedure does not entail any danger to the patient, as was demonstrated under test conditions making the conclusions undeniable[4]. Several recatheterizations performed in the present series of cases confirm these conclusions.

Perhaps the most significant point in these results is the great difference in the number of cases of bacilluria in girls and in boys. To the author it seems that this fact is of very great importance in the probable route of infection in pyelocystitis. The marked difference in incidence between the 2 sexes practically excludes the route that is common to both, namely, from the intestinal canal to the kidney via the blood stream. As between the lymphogenous or the ascending route it does not help us to differentiate.

Numerous factors probably influence the conversion of a bacilluria into a cystitis. That these may involve variations in the character of the offending organisms has been experimentally shown by Helmholz and Beeler[5]. Occasional instances in the present study suggest that the severity of the original gastro-enteric infection may, in rare instances, play a part in the grafting of a cystitis on an infected bladder.

SUMMARY

The outstanding features of this study are the extraordinary frequency of urethral and bladder contamination during gastro-intestinal diseases, and the striking differences noted between male and female infants, making it probable that the infection does not

pass from the gastrointestinal canal to the kidneys via the blood stream.

In a series of 63 infants with various gastrointestinal diseases, 38 or 60.3% showed the presence of Gram-negative bacilli in their urine. Of those in which the first and second portions of the urine were separately cultured, 21.4% showed a sufficient number of Gram-negative bacilli in the second specimens as to constitute a bacilluria.

Urethral contamination was almost 3 times as prevalent in girl babies as in boys, while actual bacilluria (specimen 2) was twice as prevalent in girls as it was in boys.

TABLE 5—SUMMARY OF CATHETERIZATIONS, ILLUSTRATING RELATIVE FREQUENCY OF BACILLURIA IN BOYS AND GIRLS.

	Total Specimens Cultured	Number Showing Gram-Bac.	Per Cent. Showing Gram-Bac.	Total Specimens Two	Number Showing Gram-Bac.	Per Cent. Showing Gram-Bac.	Number Showing Bacilluria	Per Cent. Showing Bacilluria
Boys	22	6	27.2%	15	6	40%	2	13.3%
Girls	41	32	78.0%	27	21	77.7%	7	25.9%

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Observations Regarding the Loss of Water Vapor Through the Skin in Infants

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OBSERVATIONS REGARDING THE LOSS OF WATER VAPOR THROUGH THE SKIN IN INFANTS*

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The elimination of heat from the body is dependent almost entirely on radiation, conduction and evaporation. While it is possible to determine the sum total of heat loss of an individual, the accurate determination of the parts entering into this sum is beset with difficulties which have not as yet been surmounted. Nevertheless, even relatively simple methods can yield some information, if not of the amount of heat lost by one or the other mechanism, at least about some of the conditions exercising an influence thereon. Thus, a study of the surface temperature gave us some data[1]—more accurate than available before—concerning the heat loss by conduction and radiation. In this communication we wish to report data with regard to the evaporation of water from the body surface other than the loss of water through lungs and sweat, that is, the so-called *insensible perspiration.*

The observations of Loewy,[2] made on individuals whose skin was devoid of sweat glands, would seem to demonstrate that insensible perspiration is entirely independent of the sweat glands.

Attempts to determine the amount of water eliminated from the skin in the form of insensible perspiration have been made by a considerable number of workers using various direct and indirect methods. The literature on the subject is excellently reviewed in the paper of Soderstrom and DuBois.[3] None of the direct methods seems to have been applied to the infant. In our review of the literature we were not able to find any work in which the loss of water through the skin was determined separately from that through the lungs. In infancy insensible perspiration would seem to be of special importance because the sweat glands usually do not functionate during this period of life. The determination of the insensible perspiration from the total skin surface would be most desirable. Such a procedure, if at all feasible,

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1. McClure and Sauer: Am. Jour. Dis. Child., 1915, **10,** 425.
2. Loewy: Biochem. Ztschr., 1914, **67,** 243.
3. Soderstrom and Du Bois: THE ARCHIVES INT. MED., 1917, **19,** No. 5, Part II, p. 931.

would be very difficult indeed. Some data with regard to the loss of water from a restricted area of the skin were obtained, however, by a simple method kindly suggested to us by Prof. H. G. Gale of the Department of Physics of the University of Chicago. A current of room air was drawn over an area of skin and subsequently through a set of calcium chlorid tubes properly prepared. The air current was maintained by means of a water syphoning apparatus; the rate of flow was regulated readily by measuring the water withdrawn from the syphoning bottle during a unit of time. A rectangular metal box of 4.5 cm. depth and 7.5 by 5 cm. top was placed on the skin with the open bottom enclosing an area of 37.5 square centimeters. This box was placed on the chest, and a control box of exactly the same dimensions, but closed on all sides, was placed immediately next to it. The tubes for the entrance and exit of the air were at opposite ends of the boxes and at different levels, thus insuring better ventilation. The control box was also connected with calcium chlorid tubes and syphon. By this arrangement the conditions for control and experiment were as nearly alike as possible. Temperature and humidity of the room air were also determined. The calcium chlorid tubes of the two systems were weighed before and after each observation, the control giving the water absorbed from the air; the other, the water from the air, and in addition that given off by the skin. In this way the amount of water given off by 37.5 square centimeters of skin was determined. A number of blank experiments were made to test the accuracy of our method. The difference between the two sets of tubes in the blank experiments never was as great as 1 mg.

We preferred the method described to the one employed by Galeotti and Macri.[4] Their apparatus consists of a small metal box with a sliding lid and with calcium chlorid fragments fastened to the inner surface. The box is weighed and placed on the skin and the lid withdrawn. After fifteen minutes the lid is replaced and the box again weighed. The gain in weight determines the insensible perspiration. This method is open to the objection that the apparatus is really a desiccator and, in addition, all of the carbon dioxid given off by the skin is included in the determination.

In selecting the part of the skin to be studied the chest was decided on because of the convenience of applying the metal box over this rather large and relatively flat surface; this portion of the body is also relatively immobile in the infant, in whom the abdominal type of respiration prevails. The arrangement of our experiments gives figures for only a limited area of skin. Galeotti and Macri[4] and Loewy[5] have shown that different areas of skin give off different

4. Galeotti and Macri: Biochem. Ztschr., 1914, **67**, 472.

amounts of water vapor. We cannot, therefore, with our method obtain any accurate data with reference to the total loss of water by insensible perspiration through the skin, but we can obtain information about the variations of a given area and about some factors influencing the insensible perspiration in this area.

RATE OF VENTILATION

In choosing the rate of air flow it was desired to maintain a sufficient ventilation to prevent an accumulation of moisture in the metal box overlying the skin and yet avoid a cooling effect. For our first determinations we used an air flow of 7 liters per hour. In this way the air in the box was renewed 41.5 times per hour. Later some experiments were made to see what effect a more rapid rate of ventilation would have. These determinations were made on an adult, as it is of importance that the subject be quiet during the observations. The subject was seated and wore ordinary indoor clothing. The chest

TABLE 1.—INFLUENCE OF VARIOUS RATES OF VENTILATION

No.	Room Temp., C.	Rate	No. of Renewals per Hr.	Gm. H_2O per 37.5 Sq. Cm.	Gm. H_2O* per Sq. dm. per Hr.
1	19	7 L. per 60 min	41.5	0.0137	0.0365
		14 L. per 60 min.	83.0	0.0204	0.0544
2	20	7 L. per 60 min.	41.5	0.0124	0.0331
		14 L. per 60 min.	83.0	0.0354	0.0944
3	..	7 L. per 60 min.	41.5	0.0164	0.0437
		14 L. per 60 min.	83.0	0.0247	0.0659
4	18	7 L. per 60 min.	41.5	0.0083	0.0221
		14 L per 60 min.	83.0	0.0112	0.0299
5	21	7 L. per 43 min.	58.0	0.0014	0.0052
		14 L. per 43 min.	116.0	0.0032	0.0130
6	23	7 L. per 43 min.	58.0	0.0047	0.0175
		14 L. per 43 min.	116.0	0.0169	0.0629
7	18.5	14 L. per 60 min.	83.0	0.0252	0.0672
		21 L. per 60 min.	124.5	0.0189	0.0504
8	19.5	14 L. per 53 min.	93.9	0.0356	0.1075
		28 L. per 53 min.	187.8	0.0320	0.0966
9	20	14 L. per 43 min.	116.0	0.0035	0.0130
		21 L. per 43 min.	173.6	0.0064	0.0238
10	20	14 L. per 43 min.	116.0	0.0062	0.0231
		21 L. per 43 min.	173.6	0.0085	0.0343
11	20	21 L. per 60 min.	124.5	0.0472	0.1260
		28 L. per 60 min.	166.0	0.0285	0.0760
12	20	21 L. per 43 min.	173.6	0.0097	0.0360
		28 L. per 43 min.	231.5	0.0095	0.0353
13	20	21 L. per 43 min.	173.6	0.0106	0.0394
		28 L. per 43 min.	231.5	0.0097	0.0360

* The value in grams of water per square decimeter per hour in all of our tables are given for convenience of comparison with the figures of other observers.

was uncovered sufficiently to place the boxes on the skin. Each experiment consisted of two determinations following one another with just enough time intervening for weighing the calcium chlorid tubes. Deductions can, of course, be made only from such successive determinations, as it has been shown by Loewy[2] that the amount of insensible perspiration may vary considerably in the same individual on successive days under approximately similar conditions of temperature and humidity, a fact also very well illustrated in our observations.

It is seen that an increase in the rate of ventilation up to a certain point is accompanied by an increase of the values for insensible perspiration. When the rate is increased beyond this, there is not only no further increase, but even a decrease. A vasoconstriction in consequence of cooling suggests itself as a plausible explanation for this decrease. It may be mentioned that Loewy[2] has shown a definite decrease of the insensible perspiration on cooling the skin surface.

INSENSIBLE PERSPIRATION OF THE NORMAL INFANT

Table 2 gives the results of determinations of insensible perspiration in convalescent and normal infants. In Nos. 1 to 3 the infants wore only cotton diapers and the rate of ventilation was 7 liters per hour. In Nos. 4 to 8 the babies wore cotton stockings, cotton undershirts and diapers. The undershirts were opened over the chest to allow the apparatus to come in contact with the skin. The rate of ventilation was 14 liters per forty minutes. Of the latter group, in Nos. 6c and 6d the child was restless. In 6e the child was fretful and cried at times. Nos. 7 and 8 give the results obtained from observations on the same infants serving for Nos. 2 and 3, but with the room temperatures from 5.5 to 7 degrees C. higher. The relative humidity was about the same in all observations, that is, around 30 per cent. With restlessness, with fretfulness and crying, and with increased room temperature relatively high figures were obtained for the insensible perspiration. One must be guarded in concluding that these factors actually determine an increase of insensible perspiration because of the variations which occur from day to day without any attributable cause, and the observations in the table were made on different dates. We have a number of other observations, however, which give additional evidence in support of such an effect. It happened rather frequently that an infant became fretful or cried in the course of experiments intended to test the effect of various factors on the insensible perspiration, such as the effect of clothing, etc. Such determinations, of course, had to be discarded, but invariably relatively high figures were obtained. In other words, whenever we had to deal with a restless, fretful or crying baby or whenever we had occasion to repeat an observation on any infant at a room temperature

decidedly higher than previously, the insensible perspiration was invariably greater. The sum total of our observations indicates strongly that the factors just discussed actually lead to an increase of the insensible perspiration.

TABLE 2.—BARE CHEST

A. Average Room Temperature

No.	Age, Mos.	Wt., Kg.	Rect. Temp., C.	Room Temp., C.	Rate of Ventilation	Gm. H_2O per 37.5 Sq. Cm.	Gm. H_2O per Sq. dm. per Hr.	Diagnosis	Remarks
1	4½	4	37	23.5	7 L. per 60 min.	0.0288	0.0768	Convalescent from gastro-enteritis	Quiet
2	4	4	37	23	7 L. per 60 min.	0.0262	0.0698	Convalescent from gastro-enteritis	Quiet
3a*	9	5	37	23	7 L. per 60 min.	0.0198	0.0510	Convalescent from atrophy	Quiet
3b	9	5	36.5	23	7 L. per 60 min.	0.0190	0.0506	Convalescent from atrophy	Quiet
4	7	6	36	21	14 L. per 40 min.	0.0252	0.1512	Convalescent from gastro-enteritis	Quiet
5	4	6	37	25	14 L. per 40 min.	0.0725	0.4350	Normal	Quiet
6a	10	8	37	24	14 L. per 40 min.	0.0390	0.2340	Normal	Quiet
6b	10	8	37.5	26	14 L. per 40 min.	0.0504	0.3024	Normal	Quiet
6c	10	8	37	25	14 L. per 40 min.	0.0637	0.3822	Normal	Somewhat restless
6d	10	8	37.5	24.5	14 L. per 40 min.	0.0681	0.4086	Normal	Somewhat restless
6e	10	8	37.5	26	14 L. per 40 min.	0.1325	0.7950	Normal	Very restless crying at times

B. High Room Temperature

7a	4	4	37	28.5	7 L. per 60 min.	0.0546	0.1430	Convalescent from gastro-enterit's	Quiet
7b	4	4	37	30	7 L. per 60 min.	0.0634	0.1610	Convalescent from gastro-enteritis	Quiet
8	9	5	37	29	7 L. per 60 min.	0.0356	0.0949	Convalescent from atrophy	Quiet

* In tables, letters following numerals indicate additional observations on the same individuals.

INFLUENCE OF CLOTHING

In attempting to determine what effect clothing has on the amount of water vapor given off to the surrounding air, we made determinations with the metal box placed on the clothing overlying the chest and followed these almost immediately with determinations on the

bare chest. The results obtained in these observations were quite variable.

In one adult, for instance, the amount of moisture given off through the clothing (which consisted of two layers of cotton clothing that had been worn for seven to eight hours) corresponded closely with the amount given off from the bare skin of the same region (Table 3A).

In another adult with similar clothing, which had been worn for eight hours, there were somewhat greater variations (Table 3B).

In a series of observations on infants where the clothing consisted of two layers of cotton material (the ordinary cotton hospital clothing,

TABLE 3.—Showing the Influence of Clothing

Adults

	No.	Surface	Hours Clothing on	Rate	Values	Subject and Date	Kind of Clothing
A	1	Over cloth	8	14 L. 1 hr.	0.0235	McC. 4/11/17	Two layers of cotton clothing
		Bare	....	14 L. 1 hr.	0.0246		
	2	Over cloth	7	14 L. 1 hr.	0.0185	McC. 4/12/17	Two layers of cotton clothing
		Bare	....	14 L. 1 hr.	0.0175		
	3	Over cloth	7	14 L. 1 hr.	0.0234	McC. 4/13/17	Two layers of cotton clothing
		Bare	....	14 L. 1 hr.	0.0231		
B	4	Over cloth	8	14 L. 40 min.	0.0127	S. 9/28/17	Two layers of cotton clothing
		Bare	....	14 L. 40 min.	0.0066		
	5	Over cloth	8	14 L. 40 min.	0.0082	S. 9/29/17	Two layers of cotton clothing
		Bare	....	14 L. 40 min.	0.0092		
	6	Over cloth	8	14 L. 40 min.	0.0286	S. 10/ 2/17	Two layers of cotton clothing
		Bare	....	14 L. 40 min.	0.0189		

Infants

No.	Age, Mos.	Wt., Kg.	Rect. Temp., C.	Room Temp., C.	Chest	Gm. H_2O per 37.5 Sq. Cm.	Gm. H_2O per Sq. dm. per Hr.	Rate	Diagnosis	Remarks
C	1	11	10	39	23.5	Clothed	0.0361	0.1440	14 L. 40 min	Acute otitis media	Somewhat restless
						Bare	0.0304	0.1220	....	.....	Quiet
	2a	8	4.5	37	23.5	Clothed	0.0826	0.3304	14 L. 10 min	Convalescent from gastro-enteritis	Quiet
						Bare	0.0605	0.2420	.....	.....	Quiet
	2b	8	4.5	37	24.5	Clothed	0.0693	0.2772	14 L. 10 min	.....	Quiet
						Bare	0.0488	0.1952	.....	.....	Quiet
	2c	8	4.5	37	25.5	Clothed	0.1026	0.4104	14 L. 40 min.	.....	Quiet
						Bare	0.0692	0.2768	.....	.....	Slightly fretful

worn for eight hours) there was usually somewhat more moisture given off from the clothed than from the bare chest (Table 3C).

The fact that the infants frequently became fretful, or the occurrence of slight regurgitations of stomach contents, etc., made it necessary to reject a considerable number of observations. The observations on the infant in No. 2, though she was much underweight, were selected to show the effect of clothing in the absence of restlessness and other disturbing factors.

It does not seem improbable that the greater amount of moisture given off from the clothed, as compared with the unclothed chest, where such was the case, can be accounted for by the increase of skin surface temperature[1] due to the clothing.

In order to determine whether the clothing (as used in Table 3B) itself might give up moisture, we made determinations by placing the metal box over the clothing which was lying on a glass plate. The accompanying Table 4 shows the very low values thus obtained.

TABLE 4.—Tests of Clothing Alone

No.	Surface	Rate	Values in Grams	Kind of Clothing
1	Over plate glass	14 L. 40 min	0.0040	Two layers of cotton clothing as in Table 3B
2	Over plate glass	14 L. 40 min	0.0011	Two layers of cotton clothing as in Table 3B
3	Over plate glass	14 L 40 min.	0.0019	Two layers of cotton clothing as in Table 3B
4	Over plate glass	14 L. 40 min.	0.0007	Two layers of cotton clothing as in Table 3B

It was desired, furthermore, to see how easily moisture passes through the clothing. A wire mesh was placed over a shallow vessel which was filled with water to within an inch of the top. Two layers of cotton clothing such as used in the experiments were spread on this mesh and the metal box placed over the clothing; then the determination was made of the amount of moisture given off in the first forty minutes. Thirty minutes after the completion of this determination (seventy minutes after the clothing was placed over the vessel) a similar determination was made. Table 5 shows the results of this experiment.

The metal box was placed on wire mesh over the vessel of water and a determination was made; then the two layers of clothing were placed on the mesh, the metal box placed over the clothing and another determination made. Table 6 shows the result.

TABLE 5.—Tests of Clothing Placed Over Water

Surface	Hrs. Clothing on	Rate	Values	Kind of Clothing
Over dish of water	Immediately	14 L. 40 min.	0.0745	Two layers of cotton clothing as in Table 3B
Over dish of water	After 1 hour 10 min.	14 L. 40 min.	0.0733	Two layers of cotton clothing as in Table 3B

TABLE 6.—Another Determination Over Water With and Without Clothing

Surface	Hrs. Clothing on	Rate	Values	Kind of Clothing
On wire mesh 1 in. above water; no clothing	.....	14 L. 40 min.	0.0430	
Same over clothing	Immediately	14 L. 40 min.	0.0389	Balbriggan undershirt and cotton shirt

From these observations it may be concluded that this particular clothing, when worn for a number of hours, is little if any bar to water vapor loss.

Whether or not the humidity of the room air exercises an influence we cannot say. Our data do not permit us to draw any conclusions and we therefore omitted figures for the relative humidity from our tables. Niemann[5] seems to be the only one attributing a marked effect of the humidity of the air on the insensible perspiration of water from lungs and skin, while Nuttall[6] maintains that a variation in the percentage of relative humidity of the air has very little effect. This question is by no means settled; it is of great importance, but must be left to special investigation.

ATROPHIC INFANTS

In some observations on atrophic infants we found higher values for insensible perspiration than those obtained on convalescents under approximately equal conditions of room temperature, humidity and rate of ventilation.

Niemann[5] had found the total water vapor loss (that of the skin and lungs together) greater in atrophic than in normal infants. This falls in line with the observations of Schlossmann,[7] Bahrdt and Edelstein,[8] and Murlin and Hoobler,[9] which show that the metabolism of

5. Niemann: Ergebn. der inn. Med. u. Kinderh., 1913, **11**, 32.
6. Nuttall: Arch. f. Hyg., 1895, **23**, 184.
7. Schlossmann: Ztschr. f. Kinderh., 1912-1913, **5**, 227.
8. Bahrdt and Edelstein: Festschrift, Dr. Otto Heubner, Berlin, 1913.
9. Murlin and Hoobler: Am. Jour. Dis. Child., 1915, **9**, 81.

atrophic infants proceeds at a higher level, and with the observations of McClure and Sauer[1] that atrophic infants may have a high surface temperature.

It is self-evident from the foregoing that we are as yet far from being able to assign to insensible perspiration of water from the skin of the infant the rôle it plays in the heat economy of the organism. There is no doubt that it cannot be neglected as a factor of heat elimination, and, what is perhaps of special significance, some of the data given indicate that this mechanism possesses a rather great degree of elasticity. It would seem adapted to replace some of the other means of heat elimination, if that became necessary, particularly in the non-perspiring infant.

TABLE 7.—Determinations in Atrophic Infants

No.	Age. Mos.	Wt., Kg.	Rect. Temp., C.	Room Temp., C.	Chest	Gm. H_2O per 37.5 Sq. Cm.	Gm. H_2O per Sq. dm. per Hr.	Rate	Diagnosis	Remarks
1	9	5.5	36.5	25.5	Bare	0.1284	0.3157	7 L. 60 min.	Severe atrophy	Quiet
2	2	2.5	36.0	22.0	Bare	0.0694	0.1850	7 L. 60 min.	Severe atrophy	Quiet
3a	6	3.5	35.5	23.0	Bare	0.0928	0.1474	7 L. 60 min.	Severe atrophy	Quiet
3b	6	3.5	36.0	24.0	Bare	0.0847	0.2258	7 L. 60 min.	Severe atrophy	Quiet

SUMMARY

In so far as determinations of the loss of water vapor from a restricted skin area permit of conclusions with regard to the loss of water vapor from the skin in toto, it appears:

1. The rate of ventilation has a decided effect on the amount of water vapor given off by the skin. This amount increases with the rate of ventilation up to a certain point, when it seems that a cooling effect prevents a further increase.

2. Cotton garments, such as used in our experiments, do not seem to offer any obstacle to the loss of water vapor from the skin; at least not when they have been in contact with the skin a number of hours.

3. Such factors as fretfulness, crying and increase in the room temperature increase the insensible perspiration of the infant.

4. The loss of water vapor from the skin of certain atrophic infants is relatively great.

5. The loss of water vapor from the skin seems sufficiently great to make it an important factor in the heat elimination of the infant. Furthermore, this mechanism of heat elimination seems to possess a great degree of elasticity.

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THE DETOXIFYING ACTION OF SODIUM SALT ON POTASSIUM SALT IN THE GUINEA-PIG

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The literature on the toxicity of urine contains the statement that a certain percentage of this toxicity is due to potassium chloride. What is meant may perhaps be understood best by an illustration. Suppose the intravenous injection of 100 cc. of urine at a given rate is fatal to a rabbit of a given weight. This urine contains, say, 1 per cent KCl. But it would require 125 cc. of a 1 per cent KCl solution to kill a rabbit of equal weight. The urine, therefore, contains 80 per cent of the fatal dose of KCl and it is concluded that 80 per cent of its toxicity is due to KCl. Such reasoning does seem primarily rather questionable, for potassium salts are not the only salts present in such a complicated mixture as urine, where various constituents might even depress a possible toxic action of potassium. Having in mind the experiments of J. Loeb and others on balanced salt solutions, we undertook a series of experiments with the object to test whether the presence of another salt in a solution of potassium salt exercised any influence on the toxicity of the latter, on intravenous injection in a warm-blooded animal. The experiments were done on guinea-pigs and we used preferably animals weighing approximately 200 grams. The fatal dose of KCl is given by Aaronson and Sommerfeld (1) for animals of 190 grams as 0.0125 gram. The rate of injection was in all our experiments 1 cc. per minute. Our results were as follows:

Animals weighing 1) 210, 2) 205, 3) 210, 4) 210, 5) 180, 6) 250, 7) 205 and 8) 205 grams received 2 cc. of a 1 per cent KCl solution intravenously. The first six animals died while the last

two survived without showing any symptoms. A smaller dose of KCl sometimes may prove fatal to animals of about 200 grams, just as no. 6 weighing 250 grams succumbed to a dose of 2 cc., while animals of such weight usually show little effect from that amount of KCl. All animals dying after an injection were subjected to a post mortem examination and when lesions due to disease were found, the experiments were discarded. Death appears to be due to paralysis of the respiratory centers. Very frequently the heart was found beating a considerable time after cessation of respiration.

When the injection was made with a solution containing 5 per cent NaCl in addition to the 1 per cent KCl, the result was this: Two animals of 190 and 155 grams received 2 cc.; two animals of 165 and 205 grams received 3 cc.; an animal of 195 grams 4 cc.; two animals of 195 and 200 grams 5 cc. and an animal of 205 grams 6 cc. All survived showing hardly any effect from the injection. It is evident that the addition of NaCl to a KCl solution depresses the toxicity of the latter.

Two animals weighing 193 and 185 grams received 4 cc. of a solution containing 3 per cent NaCl and 1 per cent KCl and both survived. A number of experiments were made with mixtures containing 1 or 2 per cent NaCl and 1 per cent KCl. While in both sets some animals survived, these experiments have to be discarded and for this reason: The experiments were not always accompanied by controls infected with a 1 per cent KCl solution, and in the last sets the controls survived also (nos. 7 and 8). All the other experiments cited here were made on the same day on which a control had succumbed to 2 cc. of a 1 per cent KCl solution, and the animals were of the same batch obtained from the dealer, having been kept several days before use under the same conditions. Such precautions proved by no means superfluous, when on account of the survival of the above mentioned controls, we continued our experiments with a 1.5 per cent solution of KCl. Of thirty guinea-pigs weighing 190 to 230 grams, seven survived an injection of 1.5 to 2 cc. of a 1.5 per cent KCl solution. Of these an animal of 215 grams had received 1.5 cc., two animals of 225 and 222 grams 2 cc. and four more animals

weighing 195, 198, 200, and 205 grams survived a dose of 2 cc. These last four animals were of the same batch and distinguished themselves from the other surviving animals, in that they did not show the slightest symptoms, while the other three showed a marked effect on the respiration and had severe convulsions. The rest of the animals of this batch were kept exclusively on carrots for three days while their previous diet had included bread. That is, the animals were now kept on a diet poor in NaCl. After this time two animals of 193 and 190 grams promptly succumbed to 2 cc. 1.5 per cent KCl. It is not certain though, that the change in diet alone entered into consideration, making the animals susceptible to the potassium salt. It is true, they looked well and were lively but they had not gained in weight. That a change of diet can influence the toxicity of various substances, such as of various fatty oils and of sodium tartrate has been demonstrated lately by Salant in coöperation with Bengis (2) and Swanson (3).

Twelve animals weighing from 190 to 212 grams received 2 cc. of a mixture containing 5 per cent NaCl and 1.5 per cent KCl. Of these twelve animals, three which weighed 190, 197, and 208 grams died. Of the remaining nine animals, six had no or only slight symptoms, such as irregularity of respiration and very slight convulsive seizures. These animals weighed 210, 222, 190, 195, 207, and 190 grams. Three animals of 198, 198, and 200 grams had more pronounced convulsions and the respiration was more affected, in one case the respiration became gasping.

This unequal effect recalls an observation of Loeb and Wasteneys (4) that some fundulus could live in a KCl solution when only a trace of $CaCl_2$ was added, while others required $CaCl_2$.

It would have been better if only animals of known parentage and raised under the same conditions could have been used for our experiments but that was impossible.

A guinea-pig of 210 grams survived an injection of 3 cc. of the solution, showing very little effect. Four animals of 190, 195, 205, and 205 grams received 4 cc. One died, the other three were little affected. Two animals of 205 and 208 grams received

5 cc., both had some convulsions, though not severe, and recovered rapidly.

Next we employed a solution containing 3 per cent NaCl and 1.5 per cent KCl. Five animals of 194 to 198 grams received 2 cc. Two died, of the three surviving ones, one had a short cessation of respiration, one some convulsions and one showed little effect, that is, the symptoms confined themselves to some irregularity of the respiration. Two animals weighing 196 and 198 grams survived a dose of 4 cc., one without symptoms, in the other the respiration stopped for a short time, returning with gasping and there were quite severe convulsions.

In another series of experiments the injections were made with a solution containing 10 per cent NaCl and 1.5 per cent KCl. Two animals (186 and 193 grams) received 2 cc. of this solution, and in both a short cessation of respiration occurred but no convulsions. One animal of 182 grams died. Two animals of 212 grams received 4 cc. Both had convulsions and irregularities of the respiration, but recovered quickly. The increase in the concentration of the NaCl from 5 to 10 per cent did not give any better results. Controls with 10 per cent NaCl gave the following results: An animal of 212 grams received 4 cc. in three and one-half minutes and showed some effect. Two animals of 225 and 230 grams received 5 cc. in four minutes without showing any definite symptoms, while an animal of 220 grams died from a dose of 4.5 cc. administered in five minutes.

Some interesting results were obtained on injection of 10 per cent NaCl, which deserve brief notice. A number of animals (twenty-two) varying in weight from 219 to 340 grams received from 2 to 10 cc. of 10 per cent NaCl soution The lenses of the eyes became cloudy in from ten to twenty minutes after the injection. When the animals survived, this cloudiness disappeared in the course of three to four hours. In some cases this cloudiness increased and a precipitate seemed to form in the lenses, arranged in a network. Such a lense excised and placed in water clears up entirely while a normal lens placed in a strong NaCl solution presents the same changes as seen in the living animal. The injection of strong NaCl solutions in the frog is known to be followed by a very visible effect on the red cor-

puscles. At no time after the injection of the 10 per cent NaCl solution could we observe any change in the form of the red blood corpuscles. Neither was there any very marked effect on their number in observations starting fifteen minutes after the injection and continued for several days.

A few experiments were made with a 2 per cent KCl solution. Here larger animals were used. Two animals of 245 and 269 grams survived 0.5 cc. of a 2 per cent KCl solution readily. Four animals of 260 to 275 grams succumbed to a dose of 1 and 1.5 cc. Two animals of 265 and 275 grams survived the injection of 2 cc. of a solution containing 5 per cent NaCl and 2 per cent KCl. Some animals of 250 to 225 grams received more than 2 cc. All of these died. The results of all these experiments show a marked diminution of the toxicity of KCl in the presence of NaCl, when both these substances are contained in the same solution.

The same effect became evident on a different arrangement of the experiment, where the animals received first an intravenous injection of a NaCl solution followed within a short period of time by an injection of the 1.5 per cent KCl solution either in the same vein or in the vein of the other side. In most of the experiments 5 cc. of a 5 per cent NaCl solution were given, the injection lasting four minutes. Only in the last two experiments 4 cc. of a 10 per cent NaCl solution was given at the rate of 1 cc. per minute. The results were:

WEIGHT	1.5 PER CENT KCl	TIME ELAPSED BETWEEN INJECTIONS	REMARKS
grams	*cc.*	*seconds*	
191	2.0	20	No symptoms
193	2.0	30	No symptoms
187	2.0	40	No symptoms
194	2.0	105	Short cessation of respiration. Some convulsions
212	2.0	15	Little sick
185	3.0	30	Twitching excitement. Little sick
210	3.0	30	Died
192	3.0	50	No symptoms
208	3.0	25	Short cessation of respiration. Some convulsions
200	3.0	35	Twitching. Little effect
212	3.0	30	Twitchings, irregular respiration. Little sick

Using such small animals the possibility had to be considered that intravenous injection of 5 cc. fluid could produce a very marked dilution of the blood and that this dilution was really responsible for the protective action by diminishing the concentration of the subsequently injected KCl below the fatal.

A number of control experiments were made replacing the 5 per cent NaCl solution by one of 0.55 per cent. This concentration was chosen because the determination of the Cl content of the serum of a few guinea-pigs gave this figure for NaCl. In all cases the NaCl injection was followed by one of 2 cc. of a 1.5 per cent KCl solution. The results were:

WEIGHT	TIME BETWEEN THE TWO INJECTIONS	REMARKS
grams	*seconds*	
190	40	Twitchings, short cessation of respiration; little sick, survived
190	20	Died
203	15	Died
197	15	Died after two minutes
205	30	Died after one minute
203	30	Died
212	25	Died

These results show clearly that the injection per se of 5 cc. of fluid cannot participate to any great extent in the protective action. But a 5 per cent solution of NaCl may not only increase the volume of the blood by the amount of solution injected but in addition by attracting fluid into the vessels, thus increasing the volume of blood much more than a 0.55 per cent NaCl solution. We have before us the results of Bornstein (5) who found shortly after intravenous or intracardial injection of 1 to 1.1 cc. of a 20 to 30 per cent NaCl solution in the guinea-pig a very marked increase of blood volume measured by the change in its hemoglobin content. The hemoglobin was determined with the hemometer of Sahli. Bornstein determined the hemoglobin before and after the injection and calculated the change in volume. For instance before the injection 76 per cent hemoglobin was found; after the injection 40. If the volume of blood at the

time of the first withdrawal of blood be put as 100, the volume after the second will be $\frac{76 \times 100}{40} = 190$. Bornstein found the greatest alteration after an intracardial injection of 1 cc. 30 per cent NaCl. One minute after the injection, the blood volume had increased to 200, i.e., it had doubled. We conducted similar experiments determining the hemoglobin with the Fleishl-Miescher hemometer. The 5 cc. NaCl solutions were injected in four minutes. The results are calculated according to Bornstein.

5 per cent NaCl

WEIGHT	
grams	
200	Volume of blood 100 seconds after injection: 123
207	Volume of blood 100 seconds after injection: 122
205	Volume of blood 100 seconds after injection: 126
	0.55 per cent NaCl
198	Volume of blood 100 seconds after injection: 124
208	Volume of blood 100 seconds after injection: 118
195	Volume of blood 100 seconds after injection: 132

These experiments do not permit the assumption that an injection of 5 cc. 5 per cent NaCl solution induces a markedly greater increase in blood volume than an injection of 5 cc. of a 0.55 per cent NaCl solution in the guinea-pig. It may be noted that no urine was passed during these experiments. In all our experiments with 5 per cent NaCl or less it happened but rarely that the guinea-pigs passed urine during the injection.

The two experiments now to be brought forward were made to test whether the presence of the mixture of salts in the circulation might produce a rapid diuresis and so remove sufficient KCl from the organism to render it harmless. An animal of 190 grams, after expressing the urine from the bladder received 2 cc. of a solution containing 5 per cent NaCl and 1.5 per cent KCl, with very little effect. Ten minutes after the injection about 0.1 cc. urine could be expressed from the bladder. Another animal of 210 grams with a dose of 3 cc. of the same solu-

tion also showed little effect and yielded after ten minutes about 0.2 cc. of urine. Two of the controls succumbed promptly to 2 cc. of 1.5 per cent KCl. A time of ten minutes may be regarded as ample for our purposes, for none of our animals ever died later than five minutes after a fatal dose of KCl. If an animal survived five minutes, it recovered. A hastening, therefore, of the KCl excretion cannot be accepted as a factor in the protection offered an animal by NaCl.

In the experiments reported thus far we employed salts possessing a common ion. Now the KCl was replaced by K_2SO_4. A solution of K_2SO_4, corresponding in its K content to a 1 per cent KCl solution seems to be less toxic than the latter. Thus of seven animals (weighing 230, 185, 195, 180, 209, 198, 202 grams), none succumbed to 2 cc. of an approximately 1.18 per cent K_2SO_4 solution. Two animals, weighing each 215 grams survived 3 cc. and of two others, weighing 218 and 219 grams, one survived 4 cc. With a 1.75 per cent solution of K_2SO_4, corresponding about to a 1.5 per cent KCl solution with reference to its K content, the results were as with the latter solution. Of ten animals, weighing 197 to 216 grams, three survived 1.5 or 2 cc., one of the latter animals belongs to the group described above as refractory to a 1.5 per cent KCl solution. With a solution containing 5 per cent NaCl and 1.75 per cent K_2SO_4 the results were as follows:

WEIGHT	CUBIC CENTIMETERS OF MIXTURE	REMARKS
grams		
192	2.0	Hardly any symptoms
197	2.0	Hardly any symptoms
203	3.0	Died
210	4.0	No symptoms
215	4.0	No symptoms

A solution of 2.3 per cent K_2SO_4 (about equal to 2 per cent KCl) proved fatal three times out of four in a dose of 0.5 cc. to animals weighing 205 to 237 grams and a dose of 1 cc. killed four animals from 210 to 250 grams.

The few experiments with a mixture containing 5 per cent NaCl and 2.3 per cent K_2SO_4 showed the following:

WEIGHT	CUBIC CENTIMETERS INJECTED	REMARKS
grams		
215	1.0	Died
215	1.0	Cessation of respiration, gasping, survived
205	1.0	Cessation of respiration, gasping, survived
245	2.0	Short cessation of respiration, convulsions. Survived

When the injection of 2 cc. 1.75 per cent K_2SO_4 was prec ded by 5 cc. of a 5 per cent or 0.55 per cent NaCl solution the following results were obtained:

5 per cent NaCl

WEIGHT	TIME BETWEEN THE INJECTIONS	REMARKS
grams	*seconds*	
197	15	Some twitching
198	90	Some twitching
195	15	Hardly any symptoms
187	35	Hardly any symptoms
	0.55 per cent NaCl	
196	20	Died
190	20	Died
190	20	Died

The results of the experiments with NaCl and K_2SO_4 closely resemble those with NaCl and KCl, in as much as a very marked protective influence of the NaCl becomes evident. In another series of experiments the NaCl was replaced by sodium salts. We used a Na_2SO_4 solution about equal in concentration to a 5 per cent NaCl solution with reference to its Na content. The potassium salt was KCl. A few experiments were made with a mixture containing 1 per cent KCl. Two animals of 190 and 170 grams survived an injection of 4 cc. with hardly any symptoms. Ten animals received 2 cc. of a mixture containing 6 per cent of the dry Na_2SO_4 and 1.5 per cent KCl. The weight of these

animals was: 1) 196, 2) 196, 3) 197, 4) 190, 5) 190, 6) 205, 7) 187, 8) 190, 9) 185, 10) 185. Of these animals nos. 1–5 survived, none showing much effect, while nos. 6–10 died. No. 8 died after four minutes. Some experiments were also made following an injection of 5 cc. of the Na_2SO_4 by one of 2 cc. 1.5 per cent KCl. This seemed feasible from our first experiments where two control animals of 195 and 205 grams received 5 cc. of the Na_2SO_4 solution without any effect. Later two animals of 193 to 210 grams succumbed to doses of 4 and 5 cc. But three experiments were successful, that is, the animals tolerated an injection of 5 cc. Na_2SO_4 solution without any apparent effect and the KCl could be given immediately after.

WEIGHT	TIME BETWEEN THE INJECTIONS	REMARKS
grams	*seconds*	
203	15	Not sick
214	15	Irregular respiration, some convulsions, little sick
215	20	Died

We may say that sodium sulphate can exercise a protective influence against KCl, although it does not seem to be as effective as NaCl.

Some trials were made with sodium acetate. The solution made from the salt containing 3 molecules of water of crystallization was about 11.7 per cent, corresponding about to a 5 per cent NaCl solution with reference to the Na. The arrangement of the experiment was thus: Two animals of 207 and 212 grams received 2 cc. of the sodium acetate solution without any effect. An animal of 215 grams promptly died on the injection of 2 cc. 1.5 per cent KCl. Three animals weighing 205, 207, and 212 grams received 2 cc. of a solution containing sodium acetate in the strength given and 1.5 per cent KCl. These three animals died, while two others of 210 and 212 grams weight survived the injection of 2 cc. of a mixture containing 5 per cent NaCl and 1.5 per cent KCl, without any visible effect. It seems evident even from this small number of experiments that sodium acetate cannot replace the sodium chloride in its protective action against KCl.

Desiring to test whether NaCl protected equally well against the toxic effects of other than potassium salts, the latter were replaced by ammonium chloride. First the fatal dose of this substance had to be determined. These experiments were carried out with a 2 per cent NH_4Cl solution. Animals weighing 200, 210, and 220 grams showed little effect from a dose of 1.5 cc.

Ten animals varying in weight from 190 to 230 grams received 2 cc. and only one animal weighing 220 grams recovered from a rather severe attack of intoxication, lying on its side with a gasping respiration for more than thirty minutes. The most striking lesions of a fatal dose of NH_4Cl on intravenous injection in the guinea-pig are edema of the lungs, and the presence in the lungs of multiple hemorrhages of various sizes, but as a rule rather small.

Seven animals weighing from 190 to 228 grams received 2 cc. of a mixture containing 5 per cent NaCl and 2 per cent NH_4Cl, one of 180 grams received 1.8 cc. Of these eight animals five died and the three which recovered passed though a severe attack of intoxication. The weights of these three animals were 200, 200 and 228 grams.

When an injection of 5 cc. of a 5 per cent or 0.55 per cent NaCl preceded one of 2 cc. of a 2 per cent NH_4Cl the following occurred:

5 per cent NaCl

WEIGHT	TIME BETWEEN THE INJECTIONS	REMARKS
grams	*seconds*	
205	20	Sick—recovered
202	20	Sick—recovered
197	20	Sick—recovered
202	30	Sick—recovered
	0.55 per cent NaCl	
219	25	Sick—recovered
192	25	Died
212	25	Died
207	20	Died

Three animals which survived after receiving the preliminary injection of 5 per cent NaCl were sick, lying on their side fifteen to twenty minutes and showing some hypersensitiveness on stimulation and some dyspnoea. The surviving animal of the 0.55 per cent NaCl series behaved in the same manner. The result of the experiments indicates the possibility of some protective action.

Under the conditions of our experiments, sodium salts, such as sodium chloride or sodium sulfate are capable of neutralizing the toxic action of potassium salts, such as potassium chloride or potassium sulfate, on intravenous injection in a warm blooded animal. A number of observations are recorded in which the toxic action of the potassium chloride has been counteracted by sodium chloride. Of these we may recall the experiments of Hyde (6) on Raia erinacea and binoculata, where an injection of NaCl following one of KCl quickly counteracted the effect of the latter. Garrey (7) demonstrated a detoxifying effect of NaCl on KCl in the fresh water fish Notropis blennius. The most comprehensive data on antagonist salt action, we owe to J. Loeb (8) and his co-workers. Beginning with the striking experiments of the antagonistic action of NaCl on KCl intoxication of fundulus and vice versa, J. Loeb enlarged the scope of our knowledge on antagonistic salt action, utilizing particularly the very convenient faculty of the fundulus egg to tolerate large variations in the osmotic pressure of its surroundings. These experiments put the detoxifying action of NaCl on KCl in place with the other antagonistic salt actions.

As examples of antagonistic salt action in the warm blooded animal, we may cite the detoxifying effect of KCl on NaCl observed by Joseph and Meltzer (9) and that of $MgSO_4$ on sodium oxalate, observed by Gates and Meltzer (10).

The attempt at an explanation of the results of our experiments has to take into consideration a number of factors. When the solutions of the salts are mixed before being injected, complicated reactions occur in the solution (see Smith and Ball (11)), which will exercise some influence on the result of the injection. Such reactions may also participate in the result when the injection of

one salt follows that of the other. The action of any substance is dependent on its reaching the tissues it attacks in sufficient concentration for a sufficient length of time. The failure of the KCl to kill in our experiments may be due to factors preventing it from reaching its destination in sufficient concentration for a sufficient length of time. The toxic action of KCl in the guinea pig seems to be directed chiefly against the central control of the respiration. It may be prevented from arriving there in the necessary concentration in various ways. A somewhat remote possibility exists in the influence one salt might perhaps exercise on the combination of the other with a constituent of the blood. Such a possibility is indicated by the observation of Oryng and Pauli (12), that a neutral salt ($NaNO_3$) causes a slight increase in the combining power of serum for Cl from KCl. The possibility of a hastening of the excretion of KCl by NaCl was excluded in our experiments. Another factor is to be thought of in the increase of the blood volume brought about by the injection of the hypertonic salt solutions, so that the KCl would no longer attain the necessary concentration. The experiments in which the change of blood volume was determined after the injection of 5 cc. of a 5 per cent NaCl showed a definite increase but not more than after the injection of 5 cc. of a 0.55 per cent NaCl; while the protective action of the 5 per cent NaCl solution was pronounced in contrast to that of the 0.55 per cent NaCl solution. The method of the estimation of the changes in blood volume by means of the hemoglobin determination may be none too accurate but large differences could hardly have been missed. While the increase in blood volume may be a contributory factor in the protective action afforded by the NaCl, we cannot explain our results on this basis alone. In other experiments the increase of blood volume may be of greater importance. Five guinea pigs received 2 cc. of a solution containing 50 per cent glucose and 1.5 per cent KCl. The weight of these animals was 190, 195, 195, 207, and 207 grams. All survived, showing hardly any effect. An injection of 2 cc. 50 per cent glucose is readily tolerated by guinea-pigs of such weight. In several instances the change of blood volume after the injection of 2 cc. 50 per cent glucose was

determined in the same manner as in the experiments with NaCl. The results were:

WEIGHT	
grams	
196	Increase of blood volume 35 seconds after injection to 142
198	Increase of blood volume 35 seconds after injection to 139
200	Increase of blood volume 35 seconds after injection to $166\frac{2}{3}$
200	Increase of blood volume 35 seconds after injection to 143

In these experiments the vessels of the ears were standing out very full in marked contrast to their appearance even after injections of 5 cc. 5 per cent NaCl solutions. In these cases there is a marked increase in blood volume, i.e., fluid enters the vessels rapidly as in the experiments of Bornstein with the more concentrated NaCl solutions. The rapid dilution of the blood in such cases will be of great importance provided the KCl does not leave the vessels as rapidly as the fluid enters.

In the case of NaCl there are some data indicating that the NaCl may perhaps not leave the vessels as rapidly as water enters. Dr. Eyster (13) was kind enough to tell us of some experiments in which he injected saturated NaCl solutions intravenously in dogs, determining the lowering of the freezing point. At first the blood was hypertonic but within five minutes the freezing point was normal. Eyster interprets these results as meaning, that water entering the vessels more rapidly than the salt can leave, raises the freezing point of the blood to its normal level. Nowaczek (14) injected large amounts of 10 per cent NaCl intravenously in animals and states that the concentration of NaCl in the blood was never greater than in the urine, indeed his figures for the concentration in urine exceed those for the blood quite markedly. This would speak in favor of a rapid exit of NaCl from the vessels. His tests were made in fifteen minute intervals, and from the data at our disposal, his observations do not include the happenings within the first minutes, the time which concerns us here.

The permeability of the vessel walls need not be the same for all salts and so it will be necessary to determine it separately for the

different salts. On the other side, even if the KCl should leave the vessels with the same velocity with which fluid enters, another mode of its deviation from the central nervous system is conceivable. It is known that muscles and skin are the tissues most important for the storage of water (see Engels: Die Bedeutung der Gewebe als Wasserdepots. Arch. f. expt. Pathol. u. Pharmakol., 1904, no. li, p. 346) and the muscles can give up considerable fluid on withholding water (Straub: Ueber den Einfluss der Wasserentziehung auf den Stoffwechsel und Kreislauf. Zeitschr. f. Biolog., 1899, vol. 38, p. 537). Should the tissues give up water to the blood with varying degree of ease, the KCl might chiefly go to those organs giving up their water more readily and thus be prevented from displaying its toxic action.

The absence of data precludes any further discussion of such possibilities as pointed out.

It is probable that in our experiments with NaCl and KCl the latter arrived at the central nervous system in sufficient concentration to display its toxic effect, particularly with the injection of the solution containing 5 per cent NaCl and 2 per cent KCl. Under such conditions, the fundamental experiments of J. Loeb (8) on the fundulus egg give a clew as to mechanisms by which such "antagonistic salt actions" are brought about. A comparison of our results with those of Loeb cannot be attempted at present, for we have no way of knowing the concentrations in which the salts arrive at the regions in the central nervous system where the antagonistic salt action is exercised. Besides, the differences of the routes they have to travel before reaching this destination must play a rôle, as we have pointed out above. Finally, the difference between the cells to be acted upon is too great for comparison.

We must not forget that an antagonism between NaCl and KCl has been noted by Zoethut (15) working with the gastrocnemius of the frog, where the application of NaCl previous to or simultaneous with the application of KCl hinders the development of the potassium contraction. In Hoeber's (16) experiments, Na inhibited the toxic effect of K on the skeletal muscle.

Of interest is the statement of Clowes (17):

It has been found possible, using drop systems containing a large amount of oleic acid in conjunction with Na oleate, to produce similar somewhat feeble antagonistic effects between KCl and NaCl, although usually in a NaOH system, KCl functions like NaCl as an antagonist for $CaCl_2$.

The recent observations of Loeb (18) on the formation of ionized gelatin on treatment with NaCl contain much promise for the better understanding of antagonistic salt actions.

SUMMARY

Guinea-pigs survive the intravenous injection of otherwise fatal doses of KCl and K_2SO_4 if they are administered in mixtures with NaCl.

The same effect is noted when Na_2SO_4 is added to KCl solution. The effect is, however, not quite so marked. In these experiments the concentration of the Na_2SO_4 was equal to that of the 5 per cent NaCl solution with reference to its Na content.

An equal concentration of sodium acetate does not afford any protection against the KCl intoxication.

The protection offered by NaCl against NH_4Cl is somewhat doubtful, at any rate it is much less marked than against KCl.

The presence of 50 per cent glucose in the KCl solution protects the animals very markedly.

A usual fatal dose of KCl or K_2SO_4 injected soon after an injection of 5 per cent NaCl, is tolerated, while a previous injection of 0.55 per cent NaCl does not protect, or only very rarely.

Where a previous injection of Na_2SO_4 is tolerated it may also protect against KCl.

The previous injection of NaCl seems to protect against NH_4Cl.

Several possibilities which may contribute to such protective actions, as are here described, are pointed out.

The intravenous injection of 10 per cent NaCl solution in proper dosage produces a clouding of the crystalline lens. This cloudiness appears within about twenty minutes and disappears in the course of about four hours.

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THE USE OF THICK FARINA IN THE TREATMENT OF PYLORIC STENOSIS.*

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The recent trend of the work on pyloric stenosis has been on determining whether or not the anatomic obstruction at the pylorus is of such a degree as to make the feeding of the infant an impossibility. The tendency to consider the anatomic obstruction a permanent one has been emphasized, and the fact that spasm of the pyloric ring plays a role has been neglected.

Heubner[1], Ibrahim[2], and a few others have conclusively shown that in many instances pyloric stenosis can be overcome by patiently feeding breast milk or the usual milk mixtures. The nature of the food, be it breast milk, buttermilk or skimmed milk mixtures, has apparently played a very small role in the success of any of these feeding regimes. Long intervals have been advised by some, short intervals by others. Finkelstein and Meyer[3] voice the generally accepted opinion that there is no method of feeding that will stop vomiting in pyloric stenosis. Projectile vomiting is one of the cardinal features of pyloric stenosis. The severity of the vomiting, the obstruction, the oliguria, and the rapidity of weight loss are the best indications we have of the severity of the obstruction at the pylorus.

There is another condition in infancy that is characterized by severe vomiting—the so-called neurotic vomiting in which there is no anatomic obstruction to the outflow from the stomach. In this condition Hahn[4] used 6% grits with very good success, and McClure[5] reported the cure of a case by feeding the infant thick cereal.

Whatever the mode of action of the thick cereal in neurotic vomiting there is no doubt of the beneficial results; the thickness of the cereal prevents regurgitation, and aids the normal passage of food through the pylorus into the duodenum. In 6 cases of neurotic vomiting that we have observed, the cessation of the vomiting was striking in all in which this method of feeding was tried.

The clinical results of thick cereal feeeding in neurotic vomiting was so evidently a matter of consistency that it seemed logical

* From the Otho S. A. Sprague Chicago Memorial Institute Laboratory of the Children's Memorial Hospital.

to try the same mode of treatment in pyloric stenosis. During the past 4 years it has been possible to try thick cereal feedings in 12 cases of pyloric stenosis. Three of these cases were treated by my associate, Dr. H. F. Helmholz. In all but 1 case the effect of the thick farina feedings was striking. In 11 of the 12 cases the vomiting soon stopped, although in most of the cases the peristaltic waves and the tumor (where palpable) persisted for weeks or months after the vomiting ceased. Eleven of the 12 cases are now in excellent health, the remaining 1 dying of bronchopneumonia 8 weeks after the vomiting had ceased. Of the 12 cases all but 2 were on the breast when the vomiting began. Eleven of the infants were taken care of in the home, 6 by the mother without any trained assistance. Nine of the cases occurred in boys, and 3 in girls.

Most of the infants were seen at an age of from 6 weeks to 2 months. At this time, as may readily be imagined, the feeding of a thick cereal may become quite a tedious task. Inasmuch as the consistency of the cereal seems to be the all-important factor in this treatment of pyloric stenosis, it is very necessary to use the utmost care in the preparation of the food. In order that there would be no misunderstanding of exactly how the food is to be prepared and administered, we have gone into the matter with greater detail than might appear necessary.

PREPARATION AND ADMINISTRATION OF THE THICK CEREAL.

Preparation: The proper preparation and administration of the thick farina is of great importance. It must be thoroughly cooked and sufficiently thick to adhere to an inverted spoon. One part of farina to 7 parts of fluid (3 parts of milk to 4 parts of water) boiled in a covered double boiler for an hour usually makes a cereal of proper consistency. Such a food consists of about 15% cereal. Sugar and salt may be added. Most of the cases were started on a cereal as follows, and then the proportions were changed from time to time as occasion demanded:

Skimmed milk	9 oz.
Water	12 oz.
Farina	6 tablespoons.
Lactose	3 tablespoons.

Boiled for 1 hour in a covered double boiler.

In the work it soon became evident that if the treatment was to be successful the consistency of the food required careful super-

vision. In Case 6 the cereal was not properly prepared during the first week, and there was no change in symptoms. As soon as this error was rectified the gain became rapid and the vomiting became very much less.

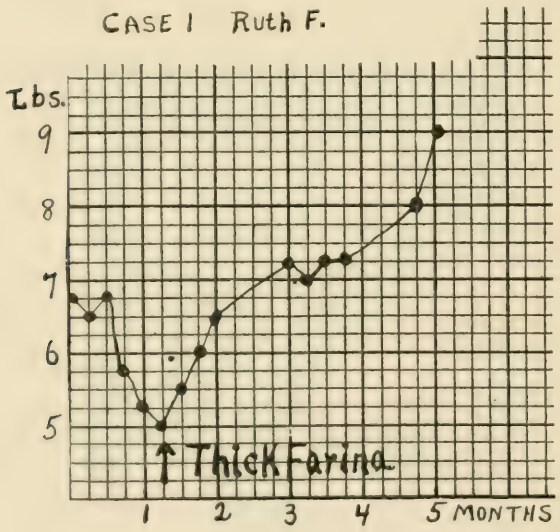
Administration: A satisfactory way of giving the food is to have the required amount of the prepared food in a cup immersed in a shallow saucepan containing hot water. By taking a little of the warm, thick cereal on the end of a narrow tongue-depressor it can very easily be placed far back into the open mouth and scraped off with another tongue-depressor. A great deal of patience is usually required during the first week as the young infant often keeps the thick cereal in the mouth for some time before swallowing. An hour or more is often required to give a feeding of a few tablespoonfuls. Two to 8 tablespoonfuls of such cereal may be given 6 or 7 times a day. In cases where breast milk is available one may try giving one or more tablespoons of the cereal before the nursing or bottle breast milk feeding. If such a regime proves unsuccessful 4 thick cereal feedings and 3 breast milk feedings may be tried for a few days. If this fails to stop the vomiting and loss of weight, then all feedings should consist of the thick farina. In very severe cases such a procedure is probably the most satisfactory. Any available breast milk can be added to the cereal provided the consistency of the food is not diminished thereby. The child should then be placed on the right side.

Case 8 illustrates the value of intelligent nursing care. After the vomiting had ceased and the child was gaining 6 to 8 ounces a week a change in nurses was attempted. The farina was not given properly or not at all, vomiting set in and the child lost 8 ounces in less than a week. At this point the nurse who had left the case again assumed charge. The vomiting stopped immediately, and the child continued to gain.

During the critical weeks of the treatment most of the infants were handled very little, and seldom taken up during or after a feeding. The child usually rested on a rather firm pillow of soft feathers. This precaution was taken to reduce to a minimum the contraction of the recti and other abdominal muscles. The child is placed on the right side for a few hours after each feeding. The stomach washings probably have some value in freeing the stomach of residual food and mucus. A 1% sodium citrate solution was used.

REPORTS OF CASES.

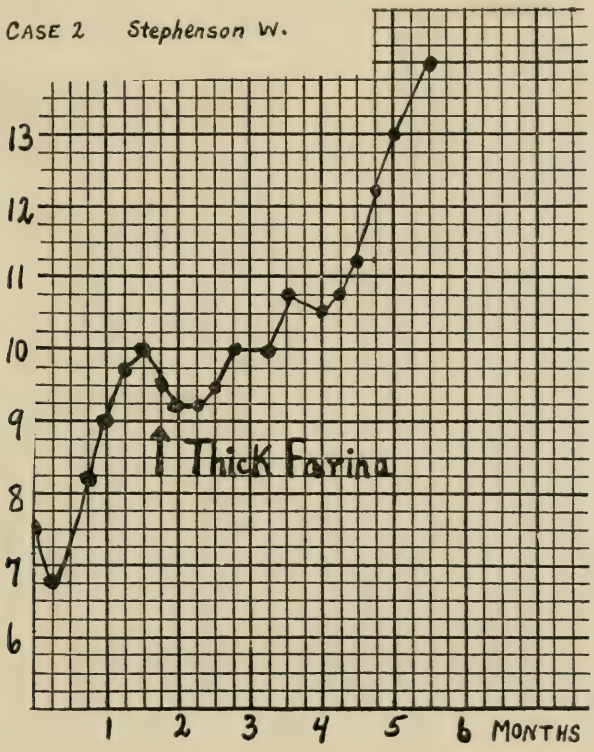
CASE 1.—Ruth F., was born August 17, 1914, and was seen the first time September 1, 1914. Her birth weight was 6 pounds and 12 ounces. She got the breast every 4 hours, and before leaving the maternity hospital at the end of the second week projectile vomiting began. When seen the first time there were projectile vomiting, peristaltic waves, and constipation. From the third to fifth weeks lavage, sodium citrate, and duodenal catheter were used without beneficial effect. By the end of the fifth week she weighed 5 pounds (a pound and twelve ounces less than at birth), and a tumor could be palpated in the pyloric region. The parents



refused to consider operative procedure, and as a last resort one-half ounce of thick farina was given just before nursings (every 3 hours). During the first week of the thick farina feedings she gained 8 ounces. The gain in weight was 8 ounces a week during the next 3 weeks. When the child was 12 weeks old the breast milk supply ceased and skimmed milk mixtures were given 3 times a day alternating with 3 thick farina feedings. The vomiting ceased almost immediately after thick farina was begun, but constipation was extreme. Starch was usually present in the stools. The peristaltic waves could still be seen after a feeding when the child was 4 months old. She had chicken-pox when 5 months old.

She is now over 3 years of age, and is in perfect health and very well nourished. None of the symptoms of pyloric stenosis have recurred.

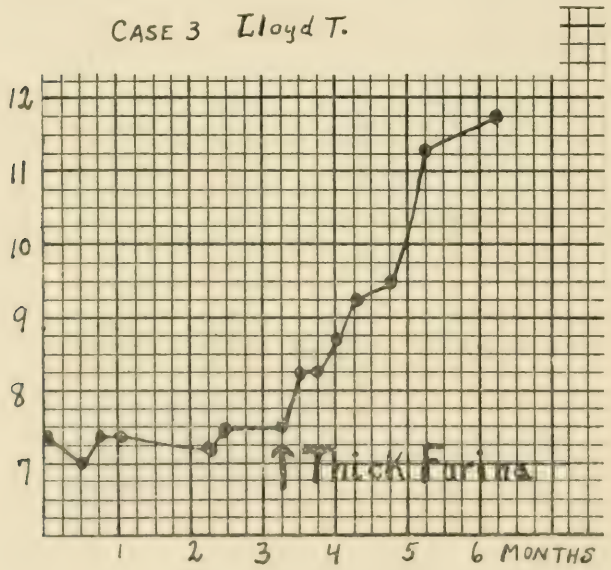
CASE 2. Stephenson W., was born January 23, 1916, and was seen for the first time about the middle of February of the same year. His birth weight was 7 pounds and 8 ounces. He was nursed



every 4 hours and gained very rapidly until the vomiting began (during the sixth week). Projectile vomiting, marked peristaltic waves, and constipation were present at this time. The epigastrium was very prominent; the hypogastrium, flat and soft. No tumor could be palpated. Lavage and duodenal catheter were resorted to a few times a week and two-thirds of an ounce of thick farina was given before nursings. The abundance of breast milk necessitated 5 to 6 minute nursings. The vomiting, constipation, and loss in weight, although less marked, continued for about 10 days. Starch was never found in the stools. A tumor

could never be felt. The weight was 10 pounds when the symptoms of pyloric obstruction began and went down to 9 pounds, but was back to 10 pounds about 5 weeks after the thick cereal was begun. There were 2 slight exacerbations of symptoms, 1 at the end of the second month, the other about the middle of the third month. The peristaltic waves were very prominent after meals for months after all other symptoms subsided, and could still be seen when the child was 7 months old. The child is in perfect health.

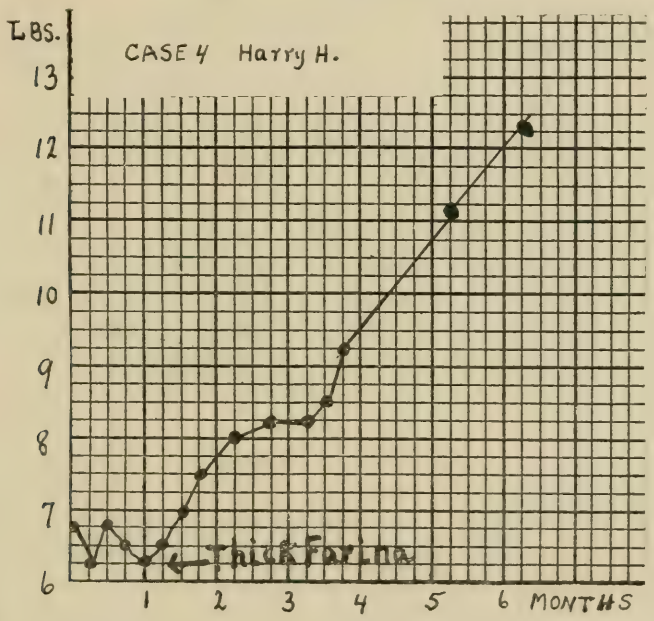
CASE 3.—Lloyd George T., was born in May, 1916. His birth weight was 7 pounds and 12 ounces. When a few weeks old projectile vomiting began with stationary weight. Nursing was dis-



continued and various "infant foods" were tried. When seen at 3 months he weighed 7 pounds 8 ounces, and there were projectile vomiting, constipation, peristaltic waves, tumor, and reduced tissue turgor. The child was given 3 thick farina feedings, alternating with 3 feedings of skimmed milk, barley water, and dextrimaltose. He gained 12 ounces during the first week and 2 pounds during the next 2 months. The peristaltic waves were present until the child was 8 months old at which time he weighed 11

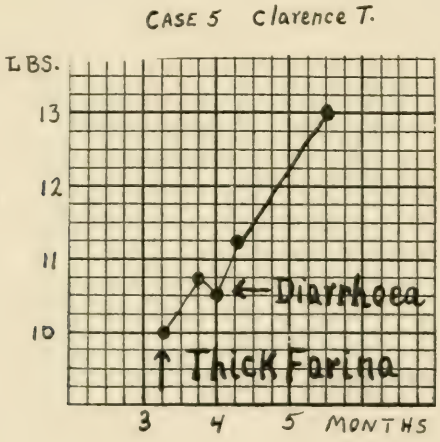
pounds. The tumor could not be felt after the first week. Starch was never found in the stools. He weighed over 20 pounds at 12 months. He is now in very good health.

CASE 4.—Harry H., was born in June, 1916. His birth weight was 6 pounds and 12 ounces. The mother had very little breast milk after the second week at which time projectile vomiting occurred several times a day. Constipation, peristaltic waves, reduced turgor, and tumor developed in the course of the next 14 days. Supplemental feedings were instituted at the end of the second week, but the weight decreased until the end of the fourth



week. At this time he weighed a little over 6 pounds. Thick farina was begun—3 such feedings alternated with the skimmed milk mixture feedings. The child immediately gained in weight, gaining a pound and twelve ounces in the first month the farina was used. The projectile vomiting had entirely disappeared for a few weeks, although the tumor and peristaltic waves were still present, when projectile vomiting again set in. The diet was rigidly enforced, lavage and great care in handling instituted, and after 2 weeks the child again gained rapidly. Starch was sometimes present in the stools. He weighed over 11 pounds at 5 months, and about 20 pounds at 1 year.

CASE 5.—Clarence T., was born in September, 1916. The birth weight was said to have been 11 pounds. Not much accurate data were obtainable. The mother said that the child never regained his birth weight. When seen he was 3½ months old and weighed 10 pounds. Tumor, peristaltic waves, projectile vomiting, constipation and emaciation were the leading symptoms. Two ounces of thick farina fed with a spoon 3 times a day, alternating with diluted evaporated milk caused a gain of 12 ounces in the

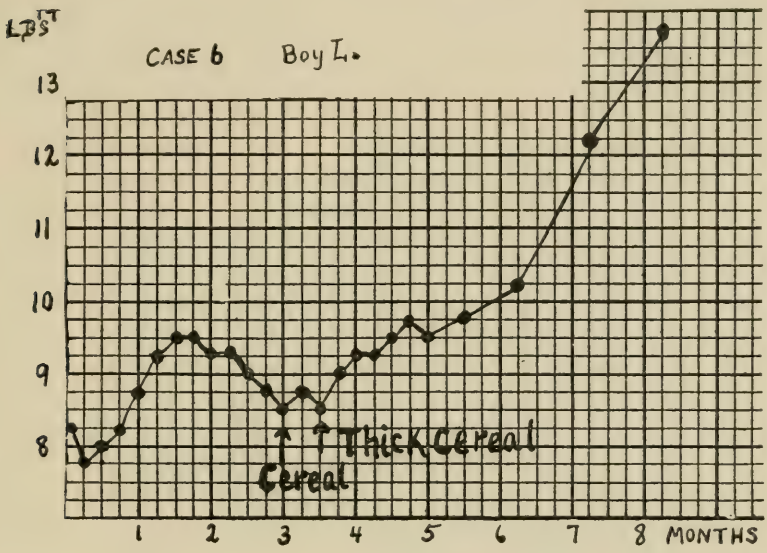


next 7 days, but the child developed a diarrhea on about the tenth day. This soon cleared up, and the gain in weight continued. The child gained 3 pounds in 2 months. The attending physician said recently that the child is very well nourished and apparently well.

CASE 6.—Nelson L., was born March 7, 1916. Projectile vomiting began when the child was 5 weeks old. It persisted until the thick barley flour feedings were given when the child was 14 weeks old. Various pediatricians had tried skimmed milk, condensed milk, lavage, etc., without any beneficial effect on the vomiting. One insisted that gastroenterostomy was necessary. The mother prepared the cereal too thinly the first week or 10 days, and the vomiting persisted, although the child gained 4 ounces. When this error was corrected by thickening the cereal the vomiting immediately became less, and the gain in weight was phenomenal. The peristaltic waves persisted for many weeks, but

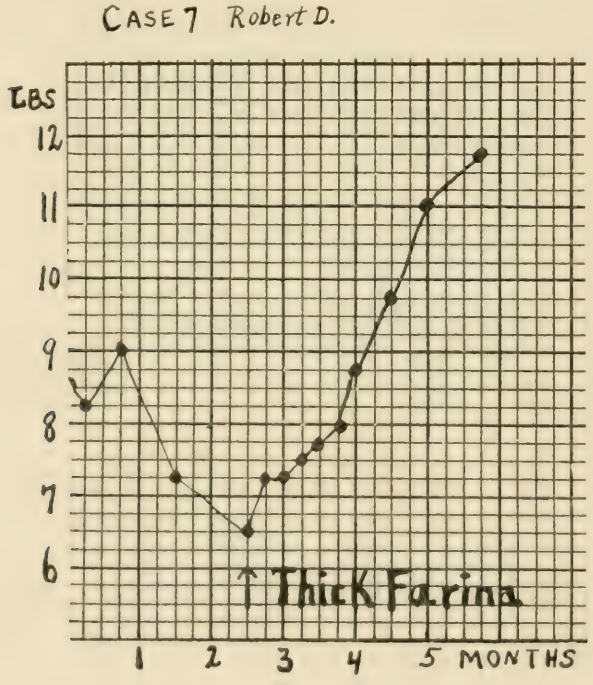
the other symptoms of pyloric stenosis soon disappeared. He weighed over 20 pounds at a year. He is now over 20 months of age and weighs over 30 pounds.

CASE 7.—Robert D., was born in August, 1915. The birth weight was 8 pounds and 8 ounces. The vomiting which began at 3 weeks, when the child weighed 9 pounds, soon became pro-



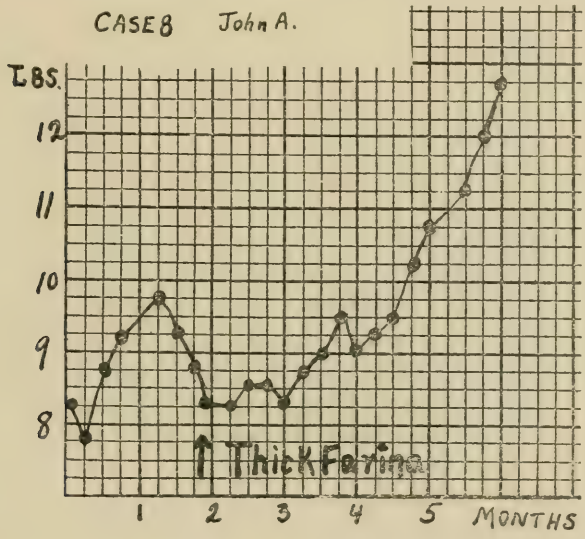
jectile and nursing was discontinued. Various "infant foods" were tried, but the loss in weight continued. When seen at 2 months there were very marked peristaltic waves, tumor, constipation, projectile vomiting, and extreme emaciation. The child weighed 6½ pounds (2 pounds less than at birth). Due to the distended epigastrium, lavage of the stomach was instituted 3 times a week for about 3 weeks. Thick farina was given 3 times a day alternated with 4 skimmed milk, barley water and dextri-maltose feedings. Although the child gained immediately (12 ounces the first week) some vomiting persisted for months. The mother said that the child would seldom lose the cereal feeding, whereas, the milk mixture was usually vomited. As starch was usually present in the hard stools it was not thought wise to give him an exclusive cereal diet. Peristaltic waves were present for at least 10 weeks after the thick farina feedings were instituted. He weighed 11 pounds at 4½ months and over 20 pounds at 12 months.

CASE 8.—John A., was born June, 1917. His birth weight was 8 pounds and 4 ounces. He was taken off the breast at 5 weeks on account of forcible vomiting. His weight had increased up to this time. When seen at 8 weeks there were rather marked peristaltic waves, projectile vomiting, subnormal temperature (96.2°F) and extreme constipation. He had lost 1½ pounds. After lavage of stomach and attempted duodenal feedings of skimmed milk mixtures had failed to check the vomiting, 3 thick cereal feedings were given to alternate with the skimmed milk mixture. The loss in weight ceased and the vomiting became



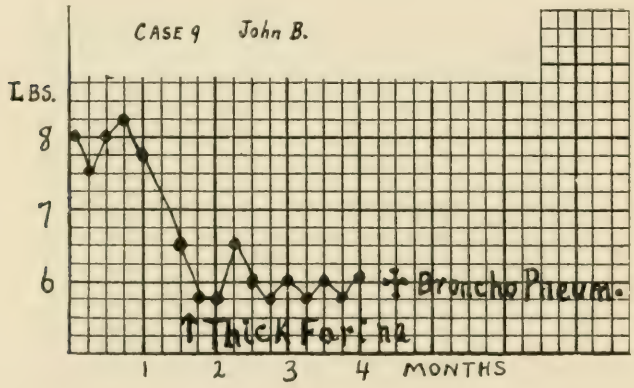
less. After the child had gained a pound he lost 8 ounces during the third month when a change in nurses was attempted. When the nurse who had taken care of him again took charge the gain in weight continued. Although peristaltic waves were still visible after a meal up to the seventh month, and there have been occasional spells of vomiting for a few days the child is gaining rapidly. The bowels are rather constipated at times. Starch was occasionally found in the stools. He is now 10 months old and weighs over 20 pounds.

CASE 9.—John B., was born in November, 1916. Projectile vomiting began during the third week. The obstetrician took them off of the breast and tried various foods. When seen at the end of the fourth week the child weighed 4 ounces less than at birth (7 pounds 12 ounces). As the mother refused to feed the young infant thick farina, skimmed milk mixtures, rich in sugars, were given with sodium citrate. The child lost a pound and four ounces in the next 2 weeks during which time the constipation became extreme, and he vomited most of his food. Peristaltic waves were very marked, but no tumor could be palpated. The parents refused to have the child operated on, or to leave him at



the hospital. They refused to give him thick farina. Diluted condensed and evaporated milks were tried for a few days but the weight continued to decrease. Finally they consented to have the child sent to the hospital. He had lost nearly 2 pounds in 3 weeks. We fed him condensed milk or breast milk 3 times a day and thick farina 4 times a day. The weight decreased to 5 pounds and 12 ounces, then went up 12 ounces in a week. It fluctuated around 6 pounds for nearly 2 months. The stomach was often washed out. There were no peristaltic waves after a few weeks, and the vomiting became very much less. During the fourth month the atrophic infant developed broncho-pneumonia from which he did not recover. No autopsy was obtainable.

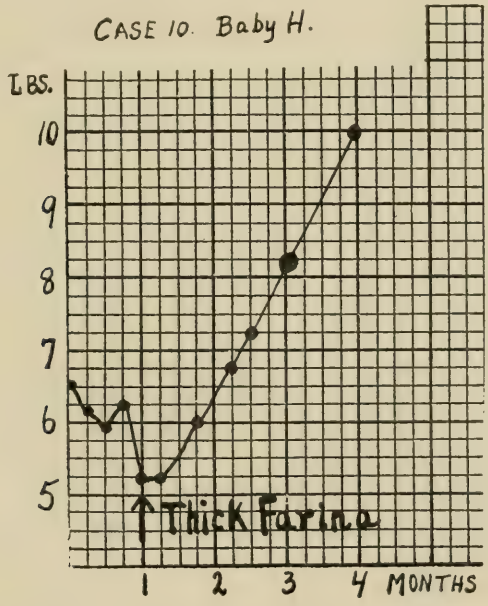
CASE 10.—Baby girl H., was born in August, 1917. Her birth weight was 6 pounds and 8 ounces. The projectile vomiting began between the second and third week while the infant was getting the breast. Because of the vomiting the child was weaned and various milk mixtures were tried without benefit. At the end of the fourth week the weight had decreased to 5 pounds and 2 ounces. When seen for the first time there were peristaltic waves and projectile vomiting, but no tumor could be palpated. The child was given from 1 to 2 ounces of thick farina every 3 hours (12 ounces of milk, 12 ounces of water, 2½ tablespoons of farina, and 1 tablespoon of sugar boiled 1 hour). At the end of 10 days there was a gain of 4 ounces. During the following 10 days she gained 10 ounces. The child has been gaining from



6 to 8 ounces a week, and the vomiting has ceased. She is now in excellent health. The peristaltic waves were visible for months, but less prominent than when the thick farina was instituted.

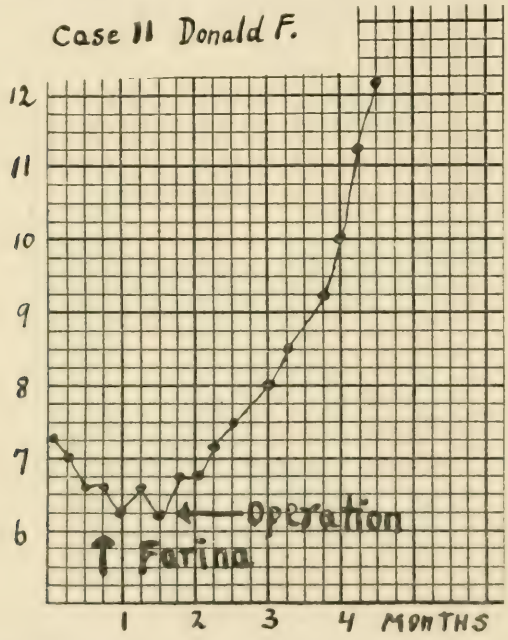
CASE 11.—Donald F., was born in December, 1917, and was seen by the author when 2 weeks old, a day after the projectile vomiting began. His birth weight was 7 pounds and 4 ounces, the breast milk supply was somewhat scant, and he had not regained his birth weight when the vomiting began. There were no peristaltic waves visible at any time, and the vomiting was very marked after each nursing. During the first few days a tablespoon or more of thick farina was given before or after the nursings without effect, then he was given 3 thick farina feedings and 4 nursings. The thick farina feedings were retained, but the nursings were always vomited. The weight remained stationary for a

week then 6 cereal feedings were given, the breast milk being added to the thick cereal. In spite of the best nursing care (the nurse who worked with Case 8), decrease in the vomiting and 1 to 3 bowel movements a day, the child failed to gain. The vomiting occurred a few times a day, but with such force that the child became very weak. He was given retention enemata containing 5% dextri-maltose in Ringer's solution several times a day for 10 days. His general condition became worse, his pulse and color bad. Peristaltic waves were never visible nor could a tumor ever be palpated. The stomach was washed with 1% sodium citrate solution several times a week. As the weight remained sta-



tionary, and he was becoming atrophic, it was thought best to have the infant operated on. The Strauss modification of the Rammstedt operation was done. Fluoroscopic examination showed that breast milk did not pass through the pylorus. The child withstood the operation remarkably well. The tumor was somewhat larger and very much harder than the one usually encountered. The lumen was much reduced. The child has been given breast milk exclusively since the operation. The mother's milk supply has developed sufficiently lately to make a wet nurse unnecessary. The child is now 4 months old, and weighs 12 pounds.

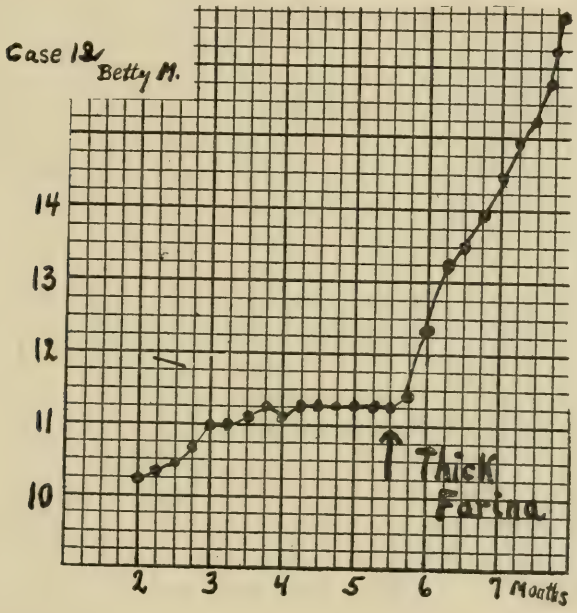
CASE 12.—Betty M., was born July 9, 1917. The birth weight was 8 pounds; her weight was 9 pounds at the end of the fourth week when spitting up began while still on the breast. She gained 1¼ pounds during the second month; her gain was 12 ounces during the third month, and the spitting up became more pronounced. During the following 2½ months there was a gain in weight of only 4 ounces when spitting up was very frequent. She spat up some of the food 4 hours after feedings, bowels were constipated and the child was weaned and various "infant foods" were tried



with no influence on the spitting up and stationary weight. At this point (at the age of 5½ months) she was brought to Evanston where a diagnosis of pyloric obstruction was made and thick farina feedings were ordered. She gained 3 ounces during the first week, and 12 ounces during each of the 2 following weeks. The spitting up became very much less, the bowels improved, and the gain in weight has been continuous ever since. She was 5½ months old and weighed 11¼ pounds when the thick cereal was begun, and at 9 months she weighed over 17 pounds. The spitting up has entirely disappeared. At no time was a tumor palpable, but peristaltic waves were still visible during the sixth month.

DISCUSSION.

The results of the thick cereal feeding in these 12 cases lead, with one exception, to very rapid subsidence of the vomiting. With the passage of the food beyond the pylorus, in practically all cases there was a rapid improvement in the nutritional condition of the child. After the thick cereal feedings had been persisted in for some time, it was found that liquid food also passed beyond the pylorus. It thus seems to be well proved that by means of the thick cereal the constriction of the pylorus is gradually loosened, so that the food will pass quite normally through the



pyloric ring. The question as to how thick cereal feedings influence the pyloric stenosis must be left open at this time.

It is naturally interesting to speculate on this action of the thick cereal. The cereal feeding that in its thickened state will be held in the stomach and passed into the duodenum will be regurgitated when thinned out with boiled water. The mere dilution with boiled water can have very little effect on the reaction of the mixture, and the resultant flow of gastric juice will probably be very nearly the same. It seems likely, therefore, that the thick, immobile cereal cannot be ejected from the stomach by the sudden

explosive contraction that produce vomiting of milk feedings. The thick cereal remaining in the stomach can be moved along by the slow peristaltic contraction of the gastric wall.

CONCLUSIONS.

Thick farina, when properly prepared and administered, brought about a cessation of vomiting in 11 of 12 cases of pyloric obstruction in young infants.

These 12 cases show that very little prognostic value in regard to the emptying time of the stomach can be attached to x-ray pictures unless taken after cereal, as well as after the ordinary milk feedings, as the patency of the pylorus is much greater after cereal feedings than it is after a milk-mixture feeding. When the x-ray is not available stomach washings 4 hours after a thick cereal feeding will serve as a valuable aid in determining the degree of obstruction and the urgency of surgical interference.

The immediate therapeutic effect of thick cereal feedings on the vomiting of pyloric stenosis warrants its thorough trial before operation is resorted to.

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THE INTRADERMAL REACTIONS TO PROTEINS OF INFANTS SUFFERING FROM GASTRO-ENTERIC DISORDERS*

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The following report is of work done on only one phase of an investigation of the skin reactions of infants and children; this particular study being concerned with the reaction of infants suffering with gastroenteric disturbances to the intradermal injection of several proteins; chiefly those of milk. Only a fraction of the work contemplated is here dealt with; the report being made at this time because of an unavoidable and indefinite interruption in the work of the writer.

During recent years considerable evidence has been advanced to show that, under certain conditions in infancy, foreign proteins may pass through the intestinal mucosa unchanged, and the assertion has also been made that such parenteral introduction may result in the sensitization of the individual to the protein concerned.

By use of precipitin tests applied to the blood serum, Ganghofer and Langer[1] found that the intestinal tract of animals less than eight days old allowed the passage of beef and egg protein when ingested in physiologic quantities. In older animals this did not occur, unless much larger quantities were given, or when the intestinal mucosa was injured. They also obtained positive tests in two infants suffering with nutritional disturbances. However, Hamburger and Sperk,[2] working with young animals, were unable to corroborate these findings.

Hayashi,[3] after feeding fifteen to twenty grams of raw egg albumin to normal infants, was unable to find the egg protein in the urine. But, on the administration of even very small quantities to infants with eczema or gastrointestinal disorders the protein was demonstrable in the urine. By precipitin tests on the urine Lawatschek[4] has shown that the gastrointestinal tract of infants under ten days of age allows foreign protein to pass unsplit, and that an appreciable quantity is excreted in the urine. Lawatschek also asserts that the permeability of the infant's in-

* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital, Chicago, Ill.

testinal mucosa to foreign protein decreases with the advance in age of the child, but increases during nutritional disturbances. Investigation that tends to corroborate the last statement has been done by Modigliana and Benini[5].

Further proof that heterologous protein may be absorbed unchanged from the intestine of infants with gastroenteric disorders, while usually not from that of the normal infant, has been obtained by use of precipitin reactions applied to the urine by Lust,[6] and Schloss and Worthen[7].

The latter authors used a precipitating serum produced by the injection of rabbits with whey proteins precipitated by saturation with ammonium sulphate.

Vaughan, Cummings and McGlumphy[8] were able to sensitize guinea-pigs to egg protein with the blood of rabbits which had been fed that protein; and Van Alstyne and Grant[9] obtained corroboratory findings with urine of dogs to whom foreign protein had been administered by means of Thiery-Villa fistulas. Lust[6] fed various forms of foreign protein to infants with nutritional disturbances, injected guinea-pigs with urine from these patients, and after three weeks obtained positive anaphylactic tests for the homologous protein.

That some degree of sensitization may result from absorbed unhydrolysed foreign proteins, is indicated by the work of Rosenau and Anderson[10] who, in feeding experiments with animals, were succesful in getting an anaphylactic response to the protein fed.* The findings of Moro,[11] and those of Bauer[12] are also very suggestive. The former made postmortem examinations of the blood of twenty-two atrophic infants, and in two found milk precipitins; while the latter demonstrated complement deviation for milk protein, as well as precipitins, in the blood of four atrophic infants.

Investigation of hypersensitiveness to milk by means of intradermal injection of milk proteins has, up to this time, been confined chiefly to eczema, asthma, hay fever, and certain cases exhibiting clinical manifestations of anaphylactic shock on the ingestion of cow's milk, and the considerable literature which has accumulated on these subjects is hardly within the scope of this paper which is concerned only with the intradermal reactions to milk proteins of infants suffering from acute or chronic gastrointestinal disturbances.

* See the literature in the paper by Kleinschmidt[13]

This study embraces, in all, the intradermal reactions of forty-three infants; twenty-six of whom were subject to gastroenteric disorders, or atrophy resulting therefrom; the remaining seventeen being patients in the hospital for some other clinical condition, and not having manifestations of digestive disturbance. Unfortunately a large number of perfectly normal breast and artificially fed infants were not available. The essential data and findings of the tests are set forth in the accompanying tables.

The egg, cow's milk and human milk albumins were all obtained by precipitation with ammonium sulphate, the per cent. of the last substance remaining in the finished product of each being determined by analysis, and in every case control injections made of a sterile ammonium sulphate solution whose concentration far exceeded that of any one of the albumin solutions. The egg protein was used as a representative of those animal proteins least likely to have been previously ingested by the infants; the milk proteins being employed for the converse reason. In a few cases blood serum from the cow was used in view of the possibility of a correlation between reactions to it and the proteins of cow's milk. The caseinogen of the cow's milk was obtained by acetic acid precipitation, twice repeated. The final neutral solution was obtained by the addition of sodium hydroxide solution. A sterile suspension of Kaolin (Merck) in freshly distilled water was used for the purpose of observing the reaction, if any, to a foreign body. It was interesting to note in this connection that while the intracutaneous injection of this substance in the adult was followed by local hyperemia and a swelling that persisted for many hours, in the infant the reaction was limited to a degree that could only be reasonably ascribed to trauma.

Solutions for injection were always used freshly prepared, and sterility insured by previous inoculation and incubation of culture media. All were used in one per cent. strength in freshly distilled water, with the exception of that of ammonium sulphate which was of five per cent.

The injections were made intracutaneously, under asceptic precautions, with a tuberculin syringe, one tenth of a cubic centimeter being given.

The term "gastroenteritis" as here employed is rather a "blanket" one, and does not refer specifically to cases exhibiting fever, vomiting, blood and pus in the stools, etc., without exception; but means rather to designate cases in which one or more of these

symptoms was present with other accompanying definite indications that the infant was suffering from well-marked digestive disturbance.

In every case following the injection of each substance used, a local redness, with more or less edema in many, developed in from a few seconds to three or four minutes. This hyperemia usually slowly increased, reaching its height in ten to fifteen minutes, at which time the extent varied from one to four centimeters in diameter. In the event of the test resulting negatively, subsidence of whatever redness and edema that was present then set in, and was complete in from twenty to thirty-five minutes. However, the behavior of a positive reaction was strikingly different. After thirty minutes, in a large majority, an area of cutaneous hyperemia with some degree of palpable edema persisted, varying from one-half to three centimeters in diameter. In a few instances the behavior up to this point was as described for a negative reaction. But then the redness reappeared about the injection site at some time during the subsequent two hours and the reaction ran thereafter the usual course. Whether the presence of the cutaneous redness was uninterrupted or not a positive reaction at the end of eighteen hours, and in many even after twenty-four hours, exhibited an area of hyperemia and palpable infiltration of from one to two centimeters in diameter. Every test recorded in the table as positive showed at the end of eighteen hours at least an area of redness and infiltration of this extent.

Kleinschmidt[13] injected intracutaneously 0.1 c.c of a Berkefeld filtrate of cow's milk inactivated at 56°. In four adults and eight children (age not given) a slight reddening of 1-2.5 cm., in diameter without swelling was noted the next day. In three adults and six children there was no reaction. After forty-eight hours there was no more visible change in the skin of any of the injected individuals. He claims that such transitory reactions occur in quite a similar manner after the injection of Ringer's solution. I have had no experience with Ringer's solution. Naturally, it is better to avoid the use of alkaline solutions for intracutaneous injections if not necessary. We may note, however, that half saturated ammonium sulphate solutions and 20 per cent. sodium chloride solutions gave not much more of a reaction than physiological saline solution. The arrangement of my observations with the rather numerous control injections leave no doubt

whatever that the reactions which were noted are of a definite significance and there is no choice but to interpret them as belonging to the group of allergic reactions.

The cases serving for our observations are arranged in two tables. The first table contains such patients which did not suffer from gastroenteritis or atrophy; the second table, those which were admitted to the hospital for intestinal disorders.

Of seventeen infants without gastroenteritis or atrophy, only five gave positive reactions to any one of the substances injected. Three of these were cases of eczema: Chas. H. having bronchitis as well; Harold D., pneumonia; while Helen had eczema alone. All three reacted positively to lactalbumin of cow's milk; one also to cow's caseinogen. Of these children two gave no history of gastroenteritis, and no claim can be made that their sensitization came about as a result of such condition, however, the possibility that they had previously absorbed unsplit foreign protein is indicated by the work of Hayashi[3] who, after administering small amounts of foreign proteins to eczematous infants, demonstrated the homologous proteins in the urine.

The fourth case, Elizabeth O., gave markedly positive reactions to lactalbumin and caseinogen of cow's milk. The baby had pneumonia, unattended by either gastroenteric disturbance or eczema. However, on inquiring more closely into her history, it was learned that three months previously, while on a modified cow's milk feeding she had undergone a severe attack of gastroenteritis.

The fifth case, Lorraine C., suffering from an acute and very severe attack of scurvy, showed, at the end of twenty-four hours, about the injection site of the lactalbumin of cow's milk, a deep bluish-red area of cutaneous reaction, measuring 2.5 centimeters in diameter, with marked infiltration and elevation of about 2 or 3 millimeters.

The remaining twelve cases gave no reaction whatever to any of the injections. In none of them was a history of previous gastroenteritis obtainable, each having been in health up to the onset of the illness for which they were brought to the hospital. The possibility that the acute illness, in most cases, accompanied by high fever, as in the cases of pneumonia may have had some inhibiting influence on the skin reactions suggests itself. However, there was nothing encountered to substantiate this.

Reactions in Conditions Other Than Gastroenteritis and Atrophy.

Name and Data	Egg Albumin 1%	Cow's Lactalbumin 1%	Cow's Caseinogen 1%	$(NH_4)_2SO_4$ 5%	Kaolin 1%	Na Cl 1%	von Pirquet	Schick	Human Lactalbumin	Sheep's Serum
Joseph H., age 17 mos. Birth wt. 5 lbs. pres. wt. 19 lbs. Breast fed 1 yr. No previous illness. Lobar pneumonia	0	0	0	0	0	0	0	+	0	
Edward R., age 1 yr. A normal breast fed baby	0	0	0	0	0	0	0	0	0	
Bruno K., age 3 yrs. A normal child save for prolapse of rectum. Breast fed 1 yr.	0	0	0	0	0	0	0	0	0	
Richard M., age 13 mos. Breast fed for 5 wks.; Cow's Milk, etc., since. Had severe attack of enteritis at age of 4 mos. Bronchitis	0	0	0	0	0	0	0	0	0	0
Virgil B., age 2 mos. Cow's milk since birth. No intestinal disturbance. Diagnosis, underfeeding.	0	0	0	0	0	0	0	0	0	0
Helen S., age 7 mos. Breast fed 3 mos.; Cow's milk since. Eczema	0	+	0	0	0	0	0	0	0	
Frank J., age 15 mos. Breast fed. Wt. 18 lbs. Lobar pneumonia	0	0	0	0	0	0	0	0	0	
Elizabeth O., age 14 mos. Wt. 17 lbs. Breast fed 9 mos. Duration of present illness 4 days. Lobar pneumonia.*	0	+	+	0	0	0	0	0		
Anna T., age 11 mos. Wt. 19 lbs. Breast fed 9 mos.; Cow's milk since. Duration of present illness ? Acute catarrhal jaundice	0	0	0	0	0	0	0	0		
Lorraine C., age 9 mos. Birth wt. 7½ lbs. pres. wt. 12 lbs. Never on breast; always on cow's milk or Mellin's food. Scurvy for 1 mo.	0	+++	0	0	0	0	0	0		
John G., age 14 mos. Birth wt. ? pres. wt. 17 lbs. Breast fed entirely. Lobar pneumonia and Otitis Media	0	0	0	0	0	0	0	0		
Harold D., age 11 mos. Wt. 16 lbs. Breast fed 6 mos.; proprietary and cow's milk since (attended by some vomiting and diarrhoea). Is rachitic and has slight eczema. Lobar pneumonia	0	+	0	0	0	0	0	0		
Donald F., age 13 mos. Birth wt. ? pres. wt. 16 lbs. Breast fed 1 mo.; since modified cow's milk. Nephritis; purpura for 7 wks.	0	0	0	0	0	0	0	0		
Pasquale P., age 6 mos. Birth wt., feeding, etc.? Sick 3 days. Lobar pneumonia. (Is from an asylum)	0	0	0	0	0	0	0	0		
Edward McM., age 9 mos. Pres. wt. 17 lbs. Entirely breast fed. Ill 3 days. Lobar pneumonia.	0	0	0	0	0	0	0	0		
Fred M., age 18 mos. Birth wt. 10 lbs. pres. wt. 22 lbs. Nursed on breast 18 mos. Duration of illness 4 days. Broncho-pneumonia	0	0	0	0	0	0	0	0		
Chas. H., age 1 yr. Wt. 16½ lbs. Feeding. Breast 2 wks.; Cow's milk etc., since. Duration of illness, 5 days. Bronchitis and eczema	0	+	+	0	0	0	0	0		

*Had attack of severe gastroenteritis at age of 11 mos.

Reactions in Gastroenteritis and Atrophy.

NAME AND DATA	Egg Albumin 1%	Cow's Lactal-bumin	Cow's Casein-ogen	$(NH_4)_2SO_4$ 5%	Kaolin 1%	Na Cl 1%	von Pirquet	Schick	Human Lactal-bumin 1%	Sheep's Serum	Beef Serum
Margaret M., age 6 mos. Birth wt. 7½ lbs. Never on breast. Cow's milk only. Atrophy.	0	+	0	0	0	0	0	0	0	0	0
Annie K., age 5½ mos. Breast fed 6 wks.; Cow's milk since. Wt. 7 lbs. Atrophy.	0	++	0	0	0	0	0	0	0	0	++
Katharine K., age 9 mos. Cow's milk only. Wt. 10 lbs. Atrophy	0	+	0	0	0	0	0	0	0	0	0
Annie P., age 1 yr. Birth wt. 6½ lbs. pres. wt. 11 lbs. Breast fed 3 mos. Cow's milk since. Atrophy	0	+	0	0	0	0	0	0	0	0	0
Mary T., age 16 mos. Birth wt. ? pres. wt. 13½ lbs. Breast fed 7 mos., then cow's milk mixtures. Acute gastroenteritis. Ill 2 wks.	0	+	+	0	0	Na Cl 5% 0	0	0			
Harold W., age 3 mos. Birth wt. 7 lbs. pres. wt. 9 lbs. Breast fed 6 wks.; since certified cow's milk. Ill 4 wks. Acute gastroenteritis. Retropharyngeal Abscess.	0	+	0	0	0	0	0	0			
Baby P., age 3 mos. Birth wt. 6½ lbs. (1 mo. premature) pres. wt. 7½ lbs. Breast fed 3 days; since cow's milk and cereals. Acute gastroenteritis	0	+	0	0	0	0	0	0			
Paul F., age 15 mos. Birth wt. 6¾ lbs. pres. wt. 19 lbs. Feeding, modified cow's milk since birth. Duration of illness 3 wks. Acute gastroenteritis	0	+	+	0	0	0	0	0			
Francis G., age 4 mos. Birth wt. 8 lbs. pres. wt. 10 lbs. Breast fed 6 wks.; cow's milk since. Enteritis. Duration of illness ?	0	+	0	0	0	0	0	0			
Joe B., age 4 mos. Wt. 9 lbs. at present. Never on breast. Cow's milk. Gastroenteritis.	0	+	0	0	0	Na Cl 1% 0	0	0	0		
Roy S., age 13 mos. Fed cow's milk. Pres. wt. 12 lbs. Atrophy-pneumonia	0	+	0	0	0	0	0	0	0		
Roy B., age 3 mos. Fed cow's milk. Gastroenteritis and Pyelitis	0	+	0	0	0	0	0	0	0		
Richard H., age 3½ mos. Fed entirely on cow's milk. Atrophy	0	0	0	0	0	0	0	0	0	0	
Hubert J., age 5 mos. Breast fed 1 mo.; Cow's milk since. Birth wt. 7 lbs. pres. wt. 7½ lbs. Atrophy.	0	+	0	0	0	0	0	+	0	0	
Edward D., age 11 mos. Pres. wt. 12½ lbs. Breast fed 2 wks.; Cow's milk dilutions since. Atrophy.	0	+	0	0	0	0	0	0	0	0	
Fannie F., age 3 mos. Birth wt. 8 lbs. pres. wt. 6¾ lbs. Breast fed 10 days; since cow's milk. Gastroenteritis, Atrophy	0	+	0	0	0	0	0		0		
Chas. D., age 2 mos. Birth wt. 8½ lbs. pres. wt. 7 lbs. Breast fed 2 wks.; since cow's milk and barley water. Gastroenteritis and Atrophy	0	+	0	0	0	0	0	0	0		
Edward K., age 6 mos. Birth wt. ? pres. wt. 10¾ lbs. Breast fed 5 wks.; Cow's milk since. Atrophy	0	+	0	0	0	0	0	0	0		
Mike K., age 3 mos. Pres. wt. 6 lbs. Feeding condensed milk only. Atrophy	0	0	0	0	0	0	0	0	0		
Theresa G., age 6 mos. Breast fed 1 mo.; since condensed and cow's milk. Ill for 5 mos. Gastroenteritis and Atrophy	0	+	0	0	0	0	0	0	0		
Edward B., age 6 mos. Wt. 10 lbs. Previous history unobtainable. Gastroenteritis.	0	+	0	0	0	0	0	0	0		
William D., age 18 mos. Birth wt. 7¾ lbs. pres. wt. ? Breast fed 3 mos.; since cow's milk, etc. Acute gastroenteritis	0	+	0	0	0	Na Cl 5% 0	0	0			
Elizabeth R., age 1 yr. Birth wt. 7 lbs. pres. wt. 15 lbs. Breast fed 11 mos.; Cow's milk since. Ill 2 wks. Gastroenteritis and Otitis Media	0	+	0	0	0	0	0	0			
William P., age 11 mos. Pres. wt. 16 lbs. Breast fed 1 mo.; since cow's milk mixtures. Gastroenteritis.	0	+	0	0	0	0	0	0			
Earl E., age 6 wks. Breast fed 1 mo.; Cow's milk since. Sick 2 wks. Vomiting and diarrhoea, congenital lues, gastroenteritis.*	0	0	0	0	0	0	0	0			
Edward M., age 3 mos. Wt. 6 lbs. Feeding cow's milk. Sick for 3 wks. Gastroenteritis and Atrophy.	0	+	+	0	0	0	0	0			

*Wassermann ++, died at age of 7 weeks.

Turning to consideration of the skin reactions in children suffering from gastroenteritis or atrophy, the striking finding is that of twenty-six infants, twenty-three gave definite intradermal reaction to lactalbumin of the cow. Three cases reacted positively to cow's caseinogen. The much greater reactivity to lactalbumin is in agreement with the observation that almost all allergic milk reactions proceed better with lactalbumin than with caseinogen. (See Versell. Zeitschr. f. Immunitäts Forschung, 1915. Vol. xxiv, p. 267).

While the above findings are too few to admit of definite conclusion, they at least suggest that sensitization to cow's milk proteins does occur in acute or chronic gastroenteric disturbance, and that such sensitization can be demonstrated by intradermal injection of small amounts of those proteins, and further that the lactalbumin is the protein to which, probably, sensitization most easily occurs.

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SEROLOGIC LOCALIZATION OF ORGANIC BRAIN LESIONS*

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During my work on dementia precox, the dialysis method of Abderhalden, slightly modified, proved to be of great value. Yet, as this method did not, for certain reasons, become very* popular, I thought it imperative to show that if used properly it is quite reliable and more than that, it can be of great value in the clinical diagnosis of obscure cases.

It seemed advisable to attempt to localize by this method those gross brain lesions which could be verified either by an operation or by necropsy, or by unmistakable clinical evidence. The technic used in all experiments was a modification of the original method based on nearly 4,000 tests, was easy to perform, less complicated and perfectly reliable. The detailed description of this modification will be found in the latter part of this article.

In the cases in which the dialysis method has been used for the purpose of a focal brain diagnosis, twenty-five have been sufficiently studied clinically or verified by operation or necropsy to be considered. The clinical diagnosis or symptoms were not known to me in most of the cases of this series. The serum was delivered for examination with only the general information that the patient suffered from some brain lesion, the location of which was not even suggested, but which I was requested to locate by serologic means. After the test was made and the diagnosis from the test was noted, it was given to the clinician for final comparison.

In the general table (Table 1) summarizing the results, the portions of the brain ("substrates") taken for the single tests are named by Roman and Arabic numbers. Figures 1 and 2[1] show the gyri which correspond to the numbers in Table 1 and in the text. Table 2 represents the subcortical structures under III and IV.

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* From the Psychopathic Hospital of Cook County.

* Part of the work reported in this paper was conducted in the Otho S. A. Sprague Memorial Institute; another part in the Memorial Institute for Infectious Diseases.

1. Figures 1 and 2 are adapted from pictures in the "Human Anatomy" of Spalteholz.

TABLE 1

Case	I	II	III	IV
1. Wm. V. 1/5/16			§ — . . . 4 . . . . . 3 . . . . . —	. . . . . 1 . . . . .
2. Mr. P. 5/8/16			. — . . . 1 . . . . . — . . . . . —	2 3 1 . . . — . . . . .
3. Mr. N. 2/18/16			. — 2 . . . — . . . . . —	. . . . 1 . . . . 2 . . . . .
4. A. F. 6/8/20 Dr. R. Wilde			1 — . . . 1 . . . — 1 . . . . . —	— — 1 — — 3 . . . . .
5. L. D. 5/18/16			— 1 2 . . . . . — 1 . . . . . —	— . . . . . ± — . . . . .
5a. L. D. 6/7/16			— ± 3 . . . ± . . . 1 2 . . . . . —	. . . . . 1 — . . . . .
6. J. F. 6/21/16 Dr. D. Lewis			— — 3 . . . 3 . . . 2 — . . . . . —	2 3 1 2 1 . . . . .
7. G. J. 1/18/17			— 2 — . . . 1 . . . 3 . . . . . —	. . 3 — . . . . .
8. Mrs. F. 5/24/17			— — 1 . . . 2 . . . . — 1 . . . . . —	— 2 — . . . . .
9. Mr. T. 8/8/17	— — — 1 1 1 — 1 — —	— 1 — — 2	— — 1 — . . . . . 3 2 1 2 ±	1 — . . — — — — . . .
10. A. G. 11/9/17. Dr. A. Heym	1 1 1 1 2 2 — — — ±	— . . — —	— . . 2 — — 2 . . — . . 1 — ± — . .	— . . 2 . . 1 . . — . . .
11. J. M. 11/24/17. Dr. G. W. Hall	3 3 3 2 1 1 — — 1 —	— 1 2 1 —	1 — — — — — — — — — — — — —	— . . . . . — — . . .
12. F. A. 12/6/17. Dr. Thor Rettedon	2 1 2 1 1 1 . . — 3 — — 1 — 1 — 1 — 1 — 3 ± — 2 2 2 . . .	1 2 1 3 1 2 — — — 1 1 1 1 . . .	2 1 — — ± — . . 1 — — — — . . 1 3	— ± — . . . . 1 — . . . .
13. S. S. 1/28/18. Dr. G. W. Hall	— 1 2 1 3 1 2 1 — ± 3 — 3 2 — ± 3 . . — — — 2 2	2 — 1 2 1 — ± — — 1	1 — — — — . . . . . — ± — — . . — —	— 2 1 — . . — 1 — —
14. Stan. G. 1/28/18. Dr. R. Hamill	1 1 1 — 1 3 — — — — — 2 1 — — — — — — — 3	2 ± — — — — — 1 — — — — — —	— 1 ± . . — . . . . . 3 — — — — ±	— 2 — . . . . 1 — 2 —
15. V. B. 2/11/18. Dr. R. Hamill	1 — 1 1 1 1 2 3 — 3 2 3 — 1 1 . . . 3 — ± . . — 1 1	1 3 — 4 — 1 — — 3 2 3 3 —	3 3 1 — 2 . . . 3 — 2 — — . . 2 —	— — — . . . . . — —
16. T. K. 5/31/17	— — — — — 1 2 — — — — —	— 1 — — 3	— — — — 2 — . . — — — — 2 — 3 1	— — — — — — — — . . .
17. C. D. 9/24/17. Dr. G. W. Hall	— 3 1 2 1 . 2 — 2 3	— 1 1 — 2	— 1 — 1 — — . . — — — 1 — — — 2	. . . . . — — — . . .
18. H. S. 10/5/17. Dr. P. Bassoe	. . . . . — . . . . . —	. . — —	. . . . . — — — — — — — — — 1 — — 2	. . 1 — — 3 3 2 . . .
19. M. O'B. 10/5/17. Dr. J. Herrick	1 — 1 — 2 — . . . . —	— . . 2 — 2	1 . . . 2 1 2 2 — — 1 3 2 1 . . 2	. . 1 3 1 1 — . . . . .
20. Mrs. H. 10/18/17. Dr. P. Bassoe	. . — — — — — — 1 —	— — — — —	— 1 2 — 3 3 1 3 1 2 — 2 — — 1	. . 3 1 . . 1 — — . . .
21. Max. K. 10/20/17. Dr. J. Grinker	— 2 1 — — 1 2 — — —	— 1 — . . —	— — 2 . . — — — — ± — — 3 2 — 3	. . — — . . — — — . . .
22. Mrs. G. 10/22/17. Dr. S. Kuh	3 2 1 3 1 1 — 1 1	1 — 1 — 1	— — — — — — 1 — — ± 1 1 3 1	. . 1 — . . — — — . . .
23. H. G. 10/23/17. Dr. G. W. Hall	— 3 2 . . 1 2 — . . 1 1	— — — — — — — — — — — — —	— 1 — — — — — — — — 1 ± 2 2	. . 3 1 . . 1 1 1 . . .
24. Mr. X. 11/9/17. Dr. G. B. Hassan	2 2 1 2 1 — — — — — — — — — —	3 2 — — — — — — — — — — —	. . — — — — — . . ± 1 . . — — — 2 2	. . — — . . . . — — . . .
25. Mr. XX. 11/16/17. Dr. A. Heym	— — — 3 3 3 1 2 — 1	. . 3 — 1 — — — — — — — —	3 1 — 3 — — . . . . 3 — 1 — — 2 —	. . — — . . — ± — . . .

* Is= Broca's convolution.
† IIIs represents the cortical part of Ammon's horn.
‡ IIIs= Island of Reil.
§ — = negative; 1 = 1 plus; 2 = 2 plus; 3 = 3 plus.

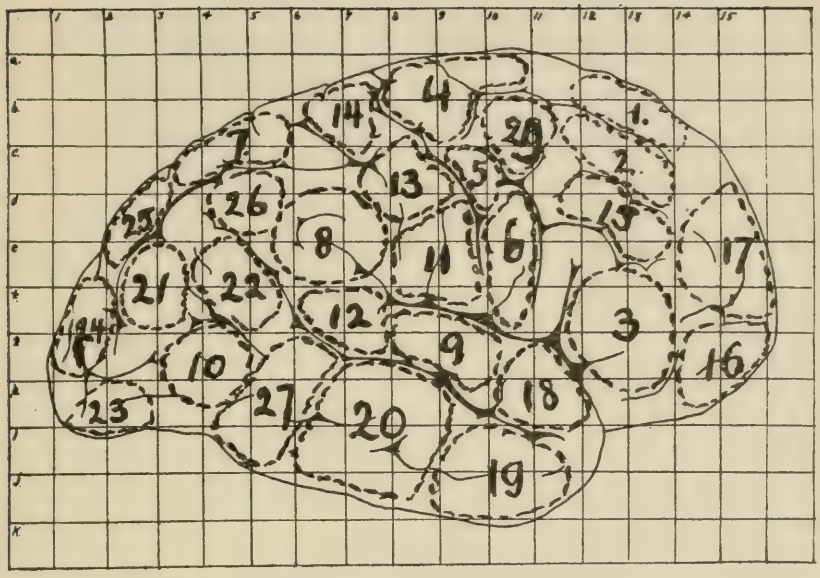

Fig. 1.—The areas of the brain cortex.

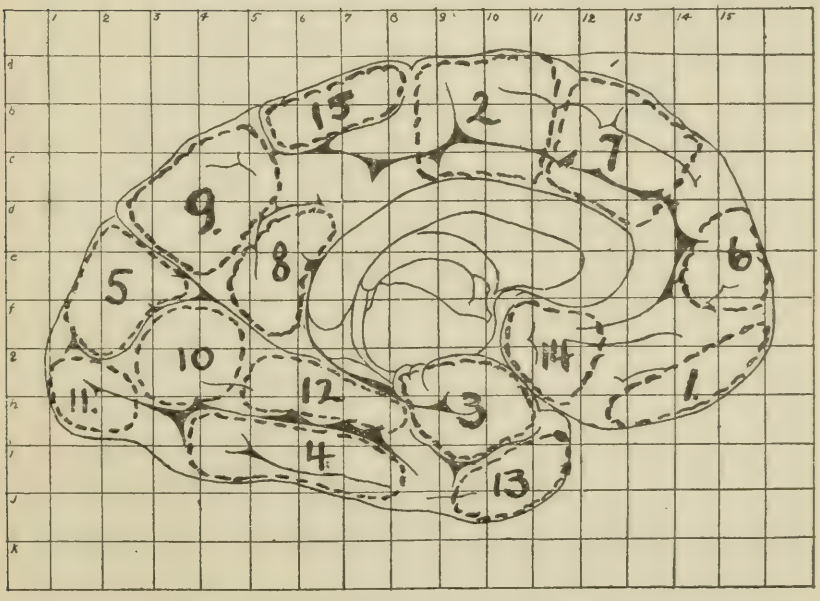

Fig. 2.—The mesial surface of the brain.

TABLE 2.—Subcortical Structures Under III and IV, Table 1

III: 1. Spinal cord, upper curvature	13. Pineal gland (human)
2. Medulla and lemniscus	14. Corpora quadrigemina
3. Pons	15. Corpus callosum
4. Corpus mammillare	IV. 1. Pineal gland (cattle)
5. Chiasma opticum	2. Pituitary (cattle, anterior)
6. Pituitary (whole, human)	3. Pituitary (cattle, posterior)
7. Olfactory tract and bulb	4. Infundibulum (cattle)
8. Fornix	5. Dura
9. Caudate nucleus	6. Cerebellar gray matter
10. Optic thalamus	7. Dentate nucleus
11. Geniculate body (lateral)	8. Internal capsule
12. Choroid plexus	9. Lentiform nucleus

APPARATUS AND TECHNIC

Dialyzers.—They are made of collodion in the following way: A 10 per cent. solution of well dried celloidin shreds in equal parts of alcohol and ether is filled into tubes ¾ by 4 inches (best with far outstanding rims; so-called fat melting point tubes). The tube is now reversed and the collodion is allowed to flow directly back into the bottle for one minute. The tube is then reversed again and allowed to stand another minute upright, until a small drop collects on the bottom of the tube. The tube is reversed again, the ring of collodion permitted to reach about the half of the height of the tube and turned back into position. Through turning of the tube the solution is now distributed evenly on the lower part of the tube. After a short experience one is able to judge just how much of the solution to let accumulate on the bottom in order to obtain a good thin tube with enough strength in the bottom to prevent folding in.

The ether vapor is blown out from the inside of the tube, which next is filled with 80 per cent. alcohol. After allowing it to stand for about twenty minutes, the alchol is poured out and the tube, with the collodion film, immersed in cool running water. After all the alcohol is washed out, which takes place after about thirty minutes, the rim is loosened, and the dialyzer can now be easily pulled out. If the dialyzer made in this way has no air bubbles and is evenly shaped, it can be used without testing. It is advisable to reject without test all those which are either uneven or too white (they ought to be just slightly milky), or if they contain in the wall, and especially on the bottom, any air bubbles. There is too much danger of breaking the very much thinner wall of air bubbles and making the dialyzer leak and consequently losing the experiment. The dialyzers are preserved in 80 per cent. alcohol and washed for half an hour in running water before every test. They can be used over and over again, only care must be taken to remove all the serum after every use. For that end it is advisable to wash them first in physiologic sodium chlorid solution to prevent any globulins from precipitating, and afterward in running water for at least one hour. They ought to be in alcohol for at least six hours between tests in order to sterilize them.

Dialyzers made in this way keep very well for many months; are ready for use at short notice, and are uniform in action.

The way of making them was adapted, with some changes, from a similar method by Pregl.[2]

Dialyzing Flasks.—Instead of the expensive Erlenmeyers with wide neck, as recommended by Abderhalden, I am using ordinary wide-mouthed, tall-form 3-ounce bottles. The advantage of these bottles is the low price, and what is more important, the possibility of utilizing a much taller column of water for dialysis, thus proportionally to the dialyzing surface increasing the rate of dialysis.

2. Pregl: Fermentforschung, 1914, **1**, 1.

Water.—Ordinary distilled water (neutral to litmus) is used.

Serum.—The blood is usually taken from any of the large veins in the arm with just enough constriction not to suppress the pulse entirely. A short needle, gage 16, was found most suitable because, while not making too big a hole in the vessel, it allows the collection of the necessary volume of blood quickly, thus preventing any annoyance to the patient. About 3 or 4 ounces of blood are collected into dry sterile bottles without the use of any anticoagulant. The blood is allowed to stand for an hour or so at room temperature, then the clot is torn to smaller pieces with sterile long hatpins and separated well from the glass, and the container left in the ice-box for several hours (over night). The serum is poured off into centrifuge tubes and centrifugated at medium speed for about ten minutes. The clear serum is pipetted off into a dry sterile graduated cylinder.

The serum should not show much hemoglobin, although that does not interfere markedly with the reaction, according to my experience. In the same way it is not essential to take blood outside of the digestive period, though it seems to give a clearer cut experiment to do so. Presence of bile in blood does not interfere with the reaction, beyond staining the dialyzers.

Substrates.—As material for my brain tissues, human brains from necropsies were used. Only brains from individuals perfectly normal, as far as the brain was concerned, were used and care was taken to use as fresh material as possible (not more than ten hours after death) to avoid postmortem changes in the tissues.

All the large vessels were removed and pieces cut out as marked in Table 2 and Figures 1 and 2. In slicing off pieces from the cortex, care was taken to remove only the gray matter, the parts from inside the fissures not being utilized at all. The pieces were cut into small particles of the size of a small pea and left stand for some time in labeled flasks filled with tap water, preferably in the ice-box. Every two hours the water was changed until all traces of blood disappeared. Now distilled water was used in the same way, and lastly distilled water with 0.5 per cent. acetic acid. After all the blood was removed from the tissue in this way, the pieces were coagulated by immersing for about two minutes in boiling water. The tissue was fished out, put back into the labeled flask and extracted cold two or three times with alcohol and as many times with ether in order to remove all fat and lipoids. When a drop of extracting fluid does not leave any fat on evaporation on a watch glass, the tissue is ready for freeing it from dialyzable substances giving the ninhydrin reaction. That is accomplished by immersing the particles in boiling water for not more than five minutes and repeating the procedure several times with fresh water. Care must be taken not to prolong the boiling too much, because then we run the danger of hydrolyzing or boiling off the specific protein. The boiling is repeated until 10 c.c. of the water in which the tissue was boiled ceases to give a reaction with 0.2 c.c. of a 1 per cent. ninhydrin solution on boiling for about three minutes; that is, until about 2 c.c. are left in the tube. The tissue is now transferred to sterile bottles, covered with boiling distilled water and about ½ inch of toluene. If the bottles are properly corked and the pieces, when necessary, taken out under aseptic conditions, the tissues thus prepared keep almost indefinitely. Just before the test the substrate is boiled three times, for a short while each, in distilled water.

Test Tubes.—The test tubes should not alkalinize the water on boiling and therefore only the best quality should be used—Jena, Nonsol, or best of all, Pyrex. The test tubes and the flasks are always cleaned with chromic acid.

The Test.—Into each of the dialyzing bottles 10 c.c. of neutral distilled water is measured with a pipet. The washed dialyzer is taken into the left (scrubbed) hand, a piece of the chosen substrate inserted into it carefully with a long forceps and from 0.7 to 1 c.c. of the clear serum added. The charged dialyzer, after being provided with a half inch layer of toluene over the

serum, is put into the bottle, a few drops of toluene dropped on the water and all covered with a watch glass. As many dialyzers are charged as different substrates are to be used and every bottle is labeled to avoid confusion. Besides those tissue dialyzers, an odd number (three or five) of serum control tubes (without any substrates) are rigged up in the same way. All bottles are left for twenty hours in an incubator regulated at between 26 and 30 C. This low temperature was chosen, as it is sufficient for the digestion, while it is not high enough to spoil the dialyzers by promoting in any very marked degree the hydrolysis of celloidin, which, being an ester, is hydrolyzible.

After twenty hours' incubation the water from the outside of the dialyzer is transferred into alkali-free test tubes. The contents are boiled for a short time to drive off the toluene, 2 drops of a 1 per cent. solution of ninhydrin are added and the contents of the tube again boiled briskly until only a ½ inch high layer is left in the test tube. If, in the case of bumping, or foaming, the liquid becomes turbid or precipitates, the test is rejected because of the possibility of the presence of protein due to a leaky dialyzer. If, on the other hand, the liquid remains clear but refuses to boil quietly, one or more short glass capillaries (sealed on the upper end) may be dropped into the tube.

The reading is made at least half an hour after the last tube is boiled, in which time the equilibrium is surely reached. The reading is made by comparison with the control tubes which sometimes are slightly colored. This may be due either to presence in the serum of digestive ferments, or amino-acids and peptones, if the blood was taken during digestion or was slightly hemolyzed. After a short experience this fact does not make any difficulties. The control tube is considered negative. Three plus was the designation given to the color which appears when 5 c.c. of a 0.1 per cent. solution of asparagin and 2 drops of the ninhydrin are added to a boiled down control tube, and the contents boiled as in all other tests. The intermediate colors are called 2 and 1 plus.

After the ninhydrin test is read the reactions are painted with colored inks —white, blue, violet, and red (3 plus)—on charts identical with Figures 1 and 2, as well as a figure indicating the subcortical structures of Table 2, but printed on transparent paper, thus showing after overimposing in transmitting light all the reactions in various levels at a time, and allowing the determination of the localization of the lesion.

RESULTS OF TESTS

Case 1.—W. V. Psychopathic Hospital, Chicago, Jan. 3, 1916. Brain tumor. Only seven different brain tissues were used as substrates. In this and the two following experiments, a mixture of all the parts of the cortex was used as substrate without differentiation. An exceedingly strong reaction with the pituitary tells the localization. Clinically, acromegaly.

Case 2.—Mr. P. Psychopathic Hospital, Chicago, Feb. 8, 1916. Brain tumor. Ten different substrates were used. The reaction with the anterior pituitary (3 plus) indicates the localization of the lesion, as would be expected, according to a clinical diagnosis of acromegaly.

Case 3.—Mr. N. Cook County Hospital, Feb. 8, 1916. Clinical Diagnosis, brain tumor. Nine different substrates were used. The cerebellar gray matter and the pons gave strong positive reactions. A necropsy performed a week later by Dr. Bissel, pathologist of Cook County Hospital, revealed a tumor in the cerebellopontine angle.

Case 4.—A. F. Presbyterian Hospital, Chicago. Dr. R. Wilder, June 8, 1916. Clinical diagnosis known to me; pineal gland disease (sexual precosity). Fourteen substrates were used. The double negative reaction with the pineal gland excludes that structure. The lesion is localized in the posterior lobe of the hypophysis (IIIb and IVa) representing probably hypofunction, which would

produce sexual symptoms similar to those due to pineal trouble. Clinical diagnosis afterward changed to posterior pituitary insufficiency.

Case 5.—L. D. Cook County Hospital. Dr. R. Hamill, May 18, 1916. Clinical findings, motor aphasia and right body hemiparesis. Eighteen different substrates were used. The most pronounced reactions are in the optic thalamus, pons, frontal cortex and the Broca convolution.

Case 5a.—Same case. June 22, 1916, shows practically the same reactions, with only slight differences in intensity of positiveness.

Case 6.—J. F. Presbyterian Hospital, Chicago. Dr. D. Lewis, June 23, 1916. Brain tumor. Fourteen different substrates used. The most important reactions are in the pituitary (III_6—3 plus) and especially its frontal lobe (IV_8—3 plus) and the occipital vision center (II_5—2 plus). Localization: The lesion is in the anterior hypophysis; visual trouble. Clinically, acromegaly; blindness.

Case 7.—G. J. Psychopathic Hospital, Chicago, April 16, 1917. Twenty-one different tissues used as brain substrates. The reaction (3 plus) with the occipital vision center (II_5), and the anterior pituitary (IV_7—3 plus) suggest the localization of the lesion in the hypophysis. A subsequent roentgen-ray examination reveals a very small sella turcica, posterior clinoid processes much enlarged and possible pituitary hypoplasia. Clinically, visual delusions.

Case 8.—Em. F. May 24, 1917. Twenty-one different substrates were used. Besides a 2 plus reaction in the pituitary (anterior), there is a prominent reaction with the visual center in the occipital lobe. The patient later gave a history of having suffered several years from double vision, but the relation of this to the reaction, if any, is not clear.

Case 9.—Mr. T. Psychopathic Hospital, Chicago, Aug. 8, 1917. Clinical diagnosis, violent mania with visual hallucinations. Forty different substrates used. The motor area of the cortex shows throughout positive reactions (I_{456} and II_2), while the positive reactions with visual path represented by the cuneus (II_5—2 plus) the corpora quadrigemina (III_{14}—2 plus) and the lateral geniculate bodies (III_{11}—3 plus) correspond with the visual delusions.

Case 10.—A. G. Dr. A. Heym, Nov. 9, 1917. Diagnosis, brain tumor. Thirty-four different substrates were used. The motor area in the cortex shows very pronounced positive reactions (I_{456}). The pituitary gives strong reactions (2 plus—III_6, IV_2). The lesion lies in the anterior lobe of the pituitary. Clinical diagnosis, acromegaly with occasional epileptiform attacks.

Case 11.—J. M. Psychopathic Hospital, Chicago, Nov. 26, 1917. Dr. G. W. Hall. Clinical diagnosis, tumor in the left frontal lobe. Thirty-four different substrates were used. The lesion was apparently cortical, because all non-cortical tissues reacted negatively (III and IV) and lies clearly in the frontal lobe (I_{123} are 3 plus positive). Because of too small a number of different parts of the frontal cortex used for the test, no nearer localization could be attempted. The clinical findings, in short, were the following: Trauma three months previously; rapid mental deterioration for the previous month; disorientation; emotionalism; recognized objects but could not name them; general tremor; jerking of right arm at times. In the beginning of February the patient died after a second trephining (in the left frontal region) and on necropsy, performed by Dr. LeCount, was found a glioma of the left parietal lobe in the neighborhood of the base of the temporal lobe.

Case 12.—F. A. Presbyterian Hospital, Chicago, Dec. 6, 1917. Dr. Thor Rothstein. Diagnosis, brain tumor. Fifty-nine different substrates were used. All the positive reactions in the frontal, central, parietal and occipital lobes could be centered by the association tracts in the temporal lobe and especially in its basal part. Here, too, were the two most pronounced reactions (I_9 and II_4). Pathology in that region would very well explain the reaction with the corpora quadrigemina (III_{14}) and the cerebellar gray matter (IV_6). It seems

that the lesion was located in the base of the temporal lobe in its white matter and that the pressure was directed mostly toward the adjoining parts of the cerebellum and possibly the corpora quadrigemina.

The clinical findings related to me after finishing the test were the following: Pupils large and equal; right does not respond to light or accommodation; left sluggishly; vision in right practically absent; distinct temporal hemianopsia in the left; no evidence of involvement of cranial nerves; rigid neck; passive motion in all directions causes pain, which is also the cause of difficulty in rising from recumbent position; vomiting; fainting spells; all reflexes normal; spinal pressure 265. Clinical diagnosis, tumor of the hypophysis. Since the patient left the hospital without being operated on, neither the clinical nor the serologic diagnosis could be confirmed.

Case 13.—S. S. Cook County Hospital, Jan. 28, 1918. Dr. G. W. Hall. Diagnosis, probably brain tumor. Fifty-nine different substrates were used. The lesion was evidently cortical, since all the most pronounced reactions appeared in the convex surface ($I_{2\,3\,6\,13\,29}$—3 plus). All of these areas could be centralized through the association fibers in I_3. Hence the localization of the lesion in the upper part of the third frontal convolution. Clinically, tumor in the right frontal lobe.

Case 14.—Stan. G. Cook County Hospital, Jan. 28, 1918. Dr. Hamill. Aphasia and right body hemiplegia. Fifty-seven different substrates were used. The 3 plus reaction with the Broca convolution suggested aphasia. Three plus reactions with the lower anterior central convolution is accounted for, on one hand, by the strong reaction with the internal capsule (2 plus—IV_8), on the other hand, by an apparent degeneration of fibers connecting the motor area with the base of the caput nuclei caudati (3 plus—III_9) and running thence through the corpus subthalamicum and the lemniscus (III_2—1 plus).

Thus the lesion, of whatever nature it is, must lie in the lower portion of the anterior central gyrus and Broca's convolution, causing aphasia, right body hemiplegia, and probably involvement of the fifth, seventh, and twelfth cranial nerves which originate in said area.

Case 15.—V. B. Cook County Hospital, Feb. 11, 1918. Dr. Hamill. Aphasia. Sixty-one different substrates were used. The reaction shows diffuse changes following pretty closely the areas supplied by the middle cerebral artery and especially its external striate branch, so that only a hemorrhage or thrombosis can be suggested without any centralized localization. Clinical diagnosis, thrombosis involving the Broca convolution.

Case 16.—T. K. Aug. 31, 1917. Cook County Hospital. The following clinical diagnosis was given to me subsequent to the completion of the test: Sudden blindness from secondary optic atrophy; probable cause, serous meningitis.

The test was performed with forty different substrates. The strongest reaction (3 plus) was with the cuneus and corpora quadrigemina, 2 plus with the optic chiasm. In short, the test was strongly positive with the visual fibers of the brain, and indicated that some pathologic process involved these fibers. This entirely corresponded with the clinical symptoms as already stated.

Case 17.—C. D. Sept. 24, 1917. Cook County Hospital. Dr. G. W. Hall. Clinical diagnosis, possible brain abscess or cerebral thrombosis.

The reactions, which were made with thirty-six different substrates, were, when positive, located almost only in the cortex and apparently scattered. The frontal lobe (I_1-I_2-I_6—see Fig. 1) proved to be strongly positive, though the corpus callosum, corpus mammillare, and the medulla reacted slightly positively. The path of the association indicated that the posterior part of the second frontal convolution was the probable seat of the lesion (point between I_2-I_{4-6}).

The necropsy performed by Dr. John Nuzum, pathologist of the Cook County Hospital, revealed a solitary abscess in exactly this place.

Case 18.—H. S. Oct. 3, 1917. Presbyterian Hospital, Chicago. Dr. P. Bassoe. Clinical diagnosis, brain tumor. The reaction was tested with forty different substrates and indicated in the first place that the lesion was not in the cortex. The most pronounced reactions were with the cerebellar gray matter (IV_6), with the dentate nucleus (IV_7), anterior pituitary and the corpus callosum. The final diagnosis from the test was: lesion in the cerebellum, which was found at the operation in the form of a large cyst of the upper frontal part of the cerebellum.

Case 19.—M. O'B. Presbyterian Hospital, Chicago. Dr. James B. Herrick. Clinical diagnosis, brain tumor. Forty substrates were used. The strongest reactions were with the posterior pituitary, with the chiasm the lateral geniculate bodies (III_{11}), the cuneus (II_5), the olfactory bulb (III_7) and the uncus (II_8).

The strong positive reaction with the optic chiasm explains the 2 plus with the vision center in the occipital lobe; especially because the reaction with the lateral geniculate body was 3 plus, thus completing the number of positive reactions throughout the central visual path. The 2 plus reaction with the olfactory apparatus, due probably to pressure from the pituitary, suggests an olfactory disturbance, a fact which would explain the 2 plus reaction with the olfactory sensory area (II_3). This suggestion is still more upheld by the 2 plus reaction with the corpus mammillare (III_4), which, as known, holds some relation to the sense of smell. The reaction with the thalamus (III_{10}) probably can be explained as involvement secondary to that of the corpus mammillare, possibly through the fasciculus thalamo-mammillare (bundle of Vicq d'Azyr). The 2 plus reaction with the choroid plexus would indicate intracranial pressure. Thus the localization of the lesion in the posterior pituitary.

After delivery of the complete report the following clinical and laboratory details were given: Diagnosis of tumor in the hypophysis; roentgen-ray examination shows great destruction in the sella turcica. Ophthalmologic examination: Visual field of left side destroyed almost completely; in the right eye very much subnormal. Almost complete loss of smell on the left side as compared with the right side.

Case 20.—Mrs. H. Oct. 18, 1917. Presbyterian Hospital, Chicago. Dr. P. Bassoe. Clinical diagnosis, brain tumor. Forty different substrates were used. The most pronounced reactions were in the fornix (III_8), the optic chiasm (III_5) and the anterior pituitary (IV_2); 2 plus reactions in the optic thalamus (III_{10}) and the choroid plexus (III_{12}), a weak reaction with the caudate nucleus (III_9), the other part of the hypophysis (IV_3), the olfactory apparatus (III_7) and the corpus callosum (III_{16}).

Since there was a general diagnosis of tumor, an attempt was made to localize it in one of the enumerated structures. A lesion in the pituitary or in the chiasm causing such strong reaction would undoubtedly cause visual disturbances and reflect on the geniculate bodies, corpora quadrigemina and the visual center in the sensory area of the occipital lobe; since these parts gave negative reactions the localization was to be in the fornix or the thalamus, and it was decided that it was most likely in the fornix, because a lesion in the thalamus probably would have brought about secondarily some cortical reactions which are missing.

After delivery of my report and localization I was informed that clinically there was no localized lesion, but an internal hydrocephalus of the third ventrical with pressure directed mostly toward its frontal wall. The reactions obtained in the test, as can be seen, are in no way contradictory to that diagnosis. Only the posterior wall of the third ventricle—the corpora quadrigemina and the pineal body—are represented by a negative reaction, while all its frontal, superior and lateral structures gave strong reactions that indicate their involvement possibly from pressure. Some time after this test the patient had a left temporal decompression performed, and since then developed signs indicating tumor in the right hemisphere.

Case 21.—M. K. Cook County Hospital, Oct. 23, 1917. Service of Dr. J. Grinker. Clinical diagnosis, tumor or abscess of the cerebellopontine angle. This clinical diagnosis was known to me when this test was made.

Thirty-eight different tissues were used. The five weak reactions in the cortex (Table 1) are in apparently no direct relation to each other and exclude the idea of a cortical lesion, but the strong reaction (3 plus) with corpus callosum (III_{15}) suggests that those reactions are bilateral. The basal ganglia, with the sole exception of the pineal gland, were negative. Thus the remaining positively reacting structure—the pons (III_5)—must be the seat of the lesion, while the 3 plus with the choroid plexus (III_{12}) indicates probably increased intracranial pressure which led to the clinical diagnosis of brain tumor. The clinical localization in the cerebellopontine angle was based chiefly on the involvement of cranial nerves from III and XII. The necropsy performed on Nov. 10, 1917, by Dr. J. Nuzum, showed pressure on the pons by a large tumor from the nasopharynx.

Case 22.—Mrs. G. Oct. 22, 1917. Michael Reese Hospital, Chicago. Dr. S. Kuh. Clinical diagnosis, brain tumor. Thirty-eight different substrates used. The whole aspect of the reaction points to two areas of possible lesion—the frontal lobe and the corpora quadrigemina. If we consider the lesion in the frontal convolution, then it would be expected that the corpora quadrigemina would be involved too, through the degeneration of corresponding association fibers, while if the primary lesion were to be in the corpora quadrigemina the reaction with the chiasm (III_5) would naturally have to be stronger than found. In addition, the prevalence of strong reactions alone points to the localization of the lesion being in the frontal lobe; probably the posterior part of the second frontal convolution.

After receiving this serologic localization, Dr. Kuh disclosed his clinical findings, which led him to the localization of the tumor in the second frontal convolution.

Case 23.—H. G. Oct. 23, 1917. Cook County Hospital. Dr. G. W. Hall. Clinical diagnosis, brain tumor. The number of positive reactions in the subcortical regions suggested the making of an attempt to localize the tumor outside of the hemispheres. On account of that, the fibers running from all cortex areas showing positive reactions were traced. The first thing which attracts the attention is the involvement of the cortical origin of the central path of cranial nerves III and XII (with the exception of the accessorius), the cortical origin of nerves V and XII is located in the lower two-thirds of the anterior central gyrus and is here represented by positive reactions (I_5 and I_6), while the oculomotor and trochlear originate in the superior portion of the second frontal convolution which gave a 3 plus reaction (I_2). The nuclei of all these nerves give a positive reaction (III_2) and it is to be remembered that the nuclei of the third and fourth cranial nerves lie nearest to the walls of the fourth ventricle and that they derive from a cortex area giving a 3 plus reaction; the corpora quadrigemina are 2 plus positive, and the centrifugal fibers from the temporal (I_9) and occipital lobe (I_{10}) run through the posterior commissure, the nearest structure to the corpora quadrigemina; the choroid plexus (III_{12}), the cerebellar gray matter (IV_6) and the dentate nucleus (IV_7) all gave a very distinct positive reaction.

On the basis of this analysis the lesion was localized in the fourth ventricle, with the involvement of the dentate nucleus and the gray matter of the cerebellum, of the aqueduct of Sylvius and of the corpora quadrigemina as far as the pineal gland. A possibility of a second focus in the hypophysis was pointed out (IV_{2a}).

The clinical diagnosis was (as disclosed after the tests were reported) tumor in the region of the lower temporal lobe of the right side, with pressure involving the corpora quadrigemina.

The necropsy performed Oct. 26, 1917, by Dr. J. Nuzum revealed a large soft glioma beginning in the dentate nucleus, and extending over the corpora quadrigemina. The hypophysis was undermined by a cyst.

Case 24.—Mr. X. Nov. 9, 1917. Private patient of Dr. B. G. Hassin. Diagnosis, well localized brain lesion. The result of the test (Table 1) made with thirty-six different substrates did not admit of any other diagnosis than that of a lesion in the upper motor area (I_{124}). The clinical diagnosis, received as usual after the delivery of the serologic report, was: Jacksonian epilepsy confined to the left leg only, due to an old skull trauma.

Case 25.—Mr. XX. Nov. 16, 1917. Private patient of Dr. A. Heym. Diagnosis, epilepsy possibly due to a localized lesion. Thirty-seven different substrates used. The test shows besides rather weak reactions in the parietal and temporal lobes a very strong reaction (3 plus) over the whole extent of the anterior central convolution (I_{4-6}) and the paracentral gyrus (II_2).

The strong positive reactions with the caudate nucleus and corpus mammillare (3 plus—III_b, IV_4) are explained by the fact that the fasciculus subcallosus, which originates in the anterior central convolution, is interrupted in the caudate nucleus, while the central cortical path of the tegmentum, which originates in the same cortical area, after crossing the caudate and lentiform nuclei passes into the neighborhood of the corpus mammillare.

On the basis of the serologic findings the lesion was localized in the upper part of the central convolution. An operation performed ten days later revealed a deep tumor of the size of a hazelnut, which could be easily palpated in this location.

In this brief report the discussion of the individual cases has been purposely omitted and only the most important and striking features have been noted. On the basis of the findings in the limited series, the serologic diagnosis is supported in four cases by the post mortem findings, in two cases by an operation, and in fifteen cases the serologic findings are in complete agreement with definite clinical evidence. Two cases differ from the clinical diagnosis, but the diagnosis has not been controlled by necropsy or operation. In one case (Case 11) the necropsy findings proved that the serologic diagnosis was erroneous.

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CHANGES IN THE BRAIN IN GAS (CARBON MONOXID) POISONING *

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This subject is important for three reasons:

1. Carbon monoxid is liberated by bursting shells and therefore is one of the gases responsible for mortality in the present war.

2. Deaths, accidental and suicidal, from inhalation of illuminating gas are increasingly frequent in civil life.[1] Henderson[2] states that carbon monoxid causes more deaths than all other gases combined. There is a reason to believe that a higher carbon monoxid content in illuminating gas is in part responsible for this. Statistics of certain cities of Great Britain[3] indicate that the higher percentage of such deaths parallels the greater proportion of water gas used in the manufacture of a cheaper product.

3. Carbon monoxid poisoning is of medicolegal significance. A history is not always available, and the characteristic cherry-red color of the blood and chemical proof of carbon monoxid are aids to diagnosis only in the first few days after poisoning. Kolisko[4] in 1914 maintained that carbon monoxid produces invariably a characteristic lesion in the brain, namely,

* From the Department of Anatomy, University of Chicago, and the Department of Pathology, Rush Medical College. This study was made under the supervision of Profs. C. J. Herrick and E. R. LeCount. The expenses were defrayed by an appropriation from a fund contributed to the University of Chicago for this purpose by twelve citizens of Chicago through the efforts of Mr. H. S. Hyman, and an appropriation made by the Otho S. A. Sprague Memorial Institute.

1. McNally, W. D.: Carbon Monoxid Poisoning, THE JOURNAL A. M. A., Nov. 10, 1917, p. 1586.
2. Henderson, Yandell: Carbon Monoxid Poisoning, THE JOURNAL A. M. A., Aug. 19, 1916, p. 580.
3. Mott, F. W.: Arch. Neurol., 1907, p. 246.
4. Kolisko, A.: Die symmetrische Encephalomalacie in dem Linsenkern nach CO-Vergiftung: Beitr. z. gerichtl. Med., 1914, **2**.

bilateral softening of the lenticular nucleus. We have attempted to discover whether this observation can be confirmed.

The present study consists of the examination of thirty-two brains obtained from the postmortem rooms of the Cook County Hospital. In every case the clinical record and complete necropsy report have been available for correlation with the pathologic condition found in the brain. The fresh brains were described on removal, hardened in 10 per cent. dilution of solution of formaldehyd, and again described. Thin cross-sections made by cutting the brain transversely at right angles to the anteroposterior axis were then described. Blocks were removed regularly, including the leptomeninges and cortex from the lateral surface of the

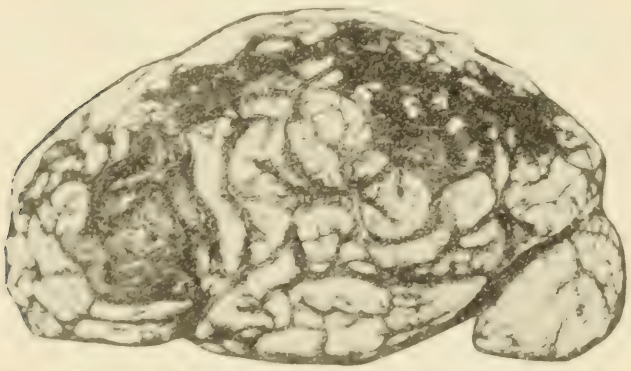

Fig. 1.—Extensive hemorrhages in the leptomeninges in gas poisoning.

hemispheres, corona radiata, centrum semiovale, corpus callosum, lenticular nucleus and internal capsule, pons, medulla and cerebellum. Other areas were selected where gross changes were evident. The blocks were dehydrated in gradually increasing strengths of alcohol, beginning with 20 per cent. in order to minimize the shrinkage of the nerve cells, embedded in paraffin and cut in sections varying from 10 to 15 microns in thickness. The stains used were hematoxylin and eosin, phosphotungstic acid hematoxylin, van Gieson's toluidin blue, Mann's methyl blue, Mallory's connective tissue, Weigert's, Marchi's, osmic acid, sudan III, and Ciaccio's.

In such a series of brains, chosen only by the one circumstance that death was due to carbon monoxid

poisoning, there were of course many pathologic changes which cannot be attributed to the gas. Thus syphilis, tuberculosis, pleurisy, pneumonia, nephritis and varying degrees of arteriosclerosis were encountered in various organs, and the picture presented by the brain tissues varied greatly according to preexisting diseases. The effort has been made to determine those changes which may be attributed to the constant factor of the carbon monoxid in the circulation.

FINDINGS

Hyperemia of the brain and leptomeninges was observed in twenty-nine cases. No diagnostic importance is attached to this common appearance in brain disease.[5] Hyperemia of other organs than the brain was frequently present.

Edema was observed in twenty-one cases. The brains do not dry rapidly as do normal brains, and when hardened they cut with more resistance. The leptomeninges are cloudy, owing to the precipitate from the edematous fluid.

Internal hydrocephalus was noted in twenty-one cases.

A cherry-red color of the blood was noticed in eleven cases, and a pink hue to the white matter of the brain in five cases. These indications of carbon monoxid in the blood may be confirmed by the spectroscopic examination and by various chemical tests,[6] which depend on the persistence of a red color to the serum or coagulum when carbon monoxid blood is mixed with certain chemicals in contrast to the gray, brown or black color of normal blood similarly treated. Of the eleven cases showing the characteristic color, four patients were found dead and five others died within a few hours of poisoning. When death is delayed for some days, these evidences of carbon monoxid poisoning are usually lacking.[4]

Petechial hemorrhages, especially in the leptomeninges and the white matter, were observed in fifteen brains. Frequently the hemorrhages were so minute that only microscopic examination served to distinguish them from the stippled appearance in cross-

5. Slavik: Etiologie Nahleho Umrti, Prague, 1912.
6. Fishbein, Morris: Illuminating Gas Poisoning, The Journal A. M. A., March 8, 1913, p. 737.

sections of hyperemic vessels. The predilection of the white matter rather than the gray is explained by the scant anastomoses in the circulation of the former. The hemorrhages are scattered through the corona radiata and the centrum semiovale, tending to be more numerous in the posterior part of the brain. The pons, medulla and brain stem are usually involved. In only one brain did hemorrhages occur in the corpus callosum. This fact deserves mention because of the statement of Mott that hemorrhages in this location are characteristic of carbon monoxid poisoning. Mott's[7] case was one of gas poisoning in the trenches,

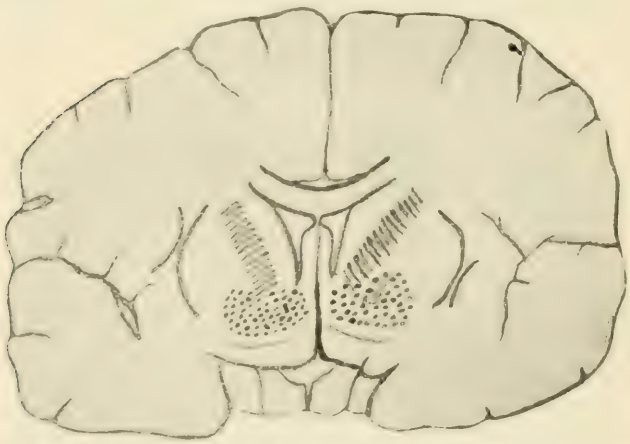

Fig. 2.—Coronal section of a brain, showing the characteristic bilateral softening of the lenticular nucleus in carbon monoxid poisoning; the dotted area represents the softening.

the history was lacking, and there is a possibility that some other gas was responsible for death. Moreover, concussion hemorrhage was not excluded. Hoover[8] has found hemorrhages in the corpus callosum in poisoning by phosgen gas ($COCl_2$) but not in carbon monoxid poisoning.

Petechial hemorrhages were not limited to the brain. Fifteen cases exhibited such hemorrhages in numerous mucous and serous membranes throughout the body, as the stomach, bowels, peritoneum, renal pelves, urinary bladder, pleura, pericardium, pharynx

7. Mott, F. W.: Brit. Med. Jour., 1917, **1**, 637.
8. Hoover: Personal communication to the authors.

and larynx and in the skin. Together with these extensive lesions of many organs besides the brain should be mentioned the occurrence in twenty-one cases of hyperplasia of the lymphoid tissue, including the spleen, thymus and tonsils, and the cervical, lingual, biliary, periaortic and intestinal lymph glands.

Arteriosclerosis of the cerebral vessels was noted in the majority of the thirty-two cases. In addition to those in which the degree of arteriosclerosis might be considered proportional to age, nineteen brains exhibited degrees of vascular degeneration excessive for their age. Ten of these patients were under 41 years of age.

Bilateral softening of the lenticular nucleus was evident on gross examination in fourteen brains. Microscopic evidence of necrosis in this region was found in each of the remaining eighteen brains. With an overwhelming dosage of gas producing death quickly, especially in young and healthy persons, gross changes may not be present. In older persons with vascular degeneration, the characteristic softening more often occurs. Age, duration of life after poisoning, amount of gas inhaled and preexisting diseases in the brain are factors influencing the amount of damage to the lenticular nucleus. One of these factors may outweigh others.

In the fourteen brains showing softening of the lenticular nucleus, serious pathologic changes due to various diseases were revealed at necropsy in each case. Ten of these persons were above 40 years of age; seven survived three days or longer; four had extensive tuberculosis; six gave evidence of syphilis aside from a degree of arteriosclerosis out of proportion to age in six others; several were chronic alcoholics. In the eighteen brains in which no gross softening of the lenticular nucleus was found, eleven brains were from persons under 40 years of age; thirteen patients died within three days. Of these, three were found dead, seven died within twenty-four hours, and one in forty-eight hours. Postmortem evidence of serious pathologic changes was much less than in the preceding group showing softening in the lenticular nucleus. The globus pallidus was invariably affected. The putamen and internal capsule were affected to a less degree. When gross softening occurred, the putamen and internal capsule were

always included. Three days in solution of formaldehyd is sufficient to bring out the difference between the soft regions and the normal tissue. In extreme cases a slushy mass occupied the position of the lenticular nucleus.

Microscopic examination confirmed the gross appearance of hyperemia, edema, capillary hemorrhages and necrosis. The striking feature of the minute changes was thrombosis of the veins. In some instances the arteries also contained thrombi. In

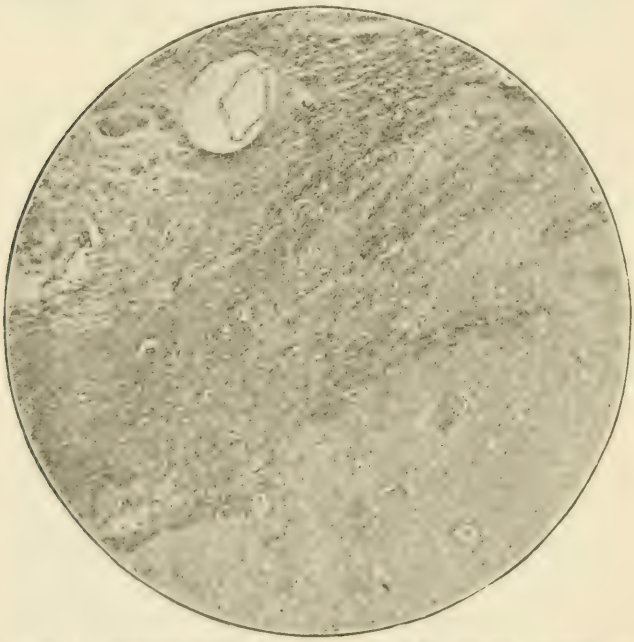

Fig. 3. Ischemic necrosis in the lenticular nucleus (upper left quadrant).

addition to fibrin masses in the vessels, many contained homogeneous, hyalin-like substances.

Stasis of the circulation without thrombus formation was indicated by agglutinated masses of red cells filling the vessels. The lenticular nucleus was almost constantly the seat of thrombosed vessels. In the six specimens in which thrombi were not seen in this location, the vessel walls were degenerated and partly or entirely digested in the midst of necrosed areas

Varying degrees of damage to the tissues about the vessels were observed. The minimum change was a slight edema. Usually necrosis was manifested by alterations in the glial nuclei, varying from pyknosis to entire disintegration. Frequently a wide region about the vessels was vacuolated and all nuclei destroyed. The arteries were usually empty. The extreme damage to the blood vessels may be in part accounted for by their previous condition, sclerotic changes being rather the rule than the exception in the cerebral vessels in this series. The capillary endothelium was usually fragmented, lying loose in the vessel lumen or altogether absent. Inflammatory changes about the vessels were seldom observed. Occasionally round cells were abundant along with a periarteritis in association with tuberculosis or syphilis in other organs, and one brain showed a polymorphonuclear accumulation. Aggregations of glial nuclei about the vessels suggest the possibility of proliferation of glial tissue, but the varying quantity of such tissue within small areas of the normal brain makes it unsafe to call these accumulations abnormal.

A conspicuous appearance in some of the brains, usually of elderly persons, was the accumulation of spherical masses from 4 to 20 microns in diameter beneath the pia and ependymal linings and about the blood vessels and perivascular lymph spaces in chaplet-like groupings. Their appearance in some regions of necrosis suggests a relationship to nerve cells. Within a small area were observed gradations from slightly altered nerve cells to granular fragments of these cells, with a faint nucleus seen only with the high power lens, to spherical homogeneous bodies. Mott[7] and Poelchen[9] have described droplets which they attributed to fatty degeneration of the vessel walls due to carbon monoxid poisoning. Other writers describe droplets of similar appearance in several types of lenticular degeneration, and numerous explanations are offered for their occurrence. They are regarded as degenerated axis cylinders, myelin sheaths and glial cells, as products of fatty degeneration of the vessel walls, and as products of the blood itself and of the cerebrospinal fluid. Whether they

9. Poelchen, R.: Zur Etiologie der Gehirnerweichung nach Kohlendunstvergiftung, Virchow's Arch. f. path. Anat., 1888, **112**.

are antemortem products or artefacts is disputed. Shionaya[10] made extensive experiments on the staining properties of neutral fats, esters of cholesterol, myelin and lipoids in the central nervous system. He concludes that these globules are normal constituents of the globus pallidus, and that sex, age and general diseases have no influence on them, but that they are

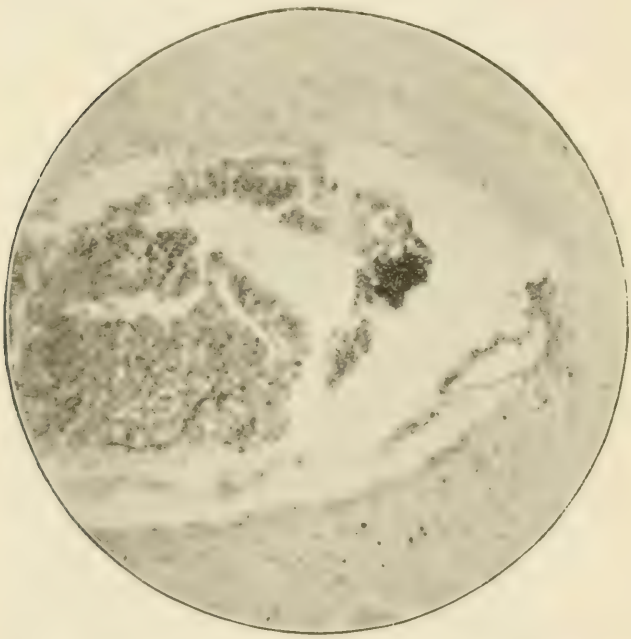

Fig. 4.—Characteristic vascular lesion in carbon monoxid poisoning. Thrombosis; degenerated vessel wall; perivascular necrosis. Phosphotungstic acid and hematoxylin stain, showing fibrin threads in blood vessel.

increased in number in all diseases producing degeneration of the nervous tissue. They stain yellow-red with sudan III, and are positive with Ciaccio's stain. The latter gives negative results with neutral fats and the esters of cholesterol. In our series the spherical bodies stained a deep purple with hematoxylin and eosin, were negative with sudan III, negative for hyalin and myelin with van Gieson's stain, and negative with Ciaccio's stain. From these staining proper-

10. Shionaya, F.: Mitt. a. d. med. Fak. d. k. Univ. z. Tokio, **14**, 121.

ties we are unable to make a definite classification. Bearing in mind the very high fat content of nervous tissue, we hesitate to attribute such accumulations to fatty degeneration of vessel walls. We presume that the spherical or globular bodies in our series are the same as those described by other authors, but we do not think they have any relation to carbon monoxid poisoning.

COMMENT

Chief interest attaches to the peculiar localization of the principal lesion in carbon monoxid poisoning in the lenticular nucleus and especially in its medial portion, the globus pallidus of both sides of the brain. Von Recklinghausen, Simon, Lesser and Poelchen observed this before Kolisko's monograph on the subject was published. Poelchen was satisfied from experiments that asphyxiation alone does not produce this lesion, but he thought that other poisons acting similarly to carbon monoxid might do so. He attributed the vulnerability of the lenticular nucleus to the long, thin end-arteries supplying this structure. Kolisko considered this explanation insufficient and called attention to the fact that the necrosis is ischemic rather than hemorrhagic. He postulated a mechanical theory, thus: The hairlike anastomosing arterioles arise from the cerebral portion of the internal carotids and their branches, the middle and anterior cerebrals. They pass at right angles from these vessels through the anterior perforated space into the brain substance. In acute poisonings the tissues supplied by these small vessels become anemic and eventually necrotic. The putamen suffers less than the globus pallidus because the vessels supplying the former have some anastomoses with a separate vascular supply. The initial increase of blood pressure in carbon monoxid poisoning tends to force the carotids upward against the brain and outward into the sylvian fissures and thus draws the small ganglionic branches outward from their proper location, which interferes with their circulation. Subsequent diminished blood pressure further lessens their blood supply. Deleterious effects of carbon monoxid on the vessel endothelium add to the retardation of the circulation. Another factor is the swelling of the brain tissue from edema pressing on the blood vessels. Again the widened carotids and

their branches press the cerebrospinal fluid against the anterior perforated space and compress the vessels which pass through this space into the basal ganglions. While not altogether convincing, this is perhaps the most satisfactory explanation yet offered for the constant involvement of the globus pallidus.

The engorgement of the vessels of the white matter and their rupture logically follow as compensatory phenomena when the circulation just described has been greatly impeded. Greenacre,[11] in a study of

Fig. 5. Accumulation of spherical bodies about a blood vessel.

spontaneous cerebral hemorrhages, calls attention to pontile hemorrhages found secondary to hemorrhages in the internal capsule and basal ganglions. She refers to anatomic peculiarities of the vessels supplying the pons (small, short, terminal branches given off at almost right angles from a large trunk), and suggests also a disturbance of blood pressure in the circle of Willis which may act on the posterior branches, as

11. Greenacre, Phyllis: Bull. Johns Hopkins Hosp., 1917, **28**, 86.

Kolisko describes in the case of the more anterior ganglionic branches.

It seems certain that the blood vessels bear the brunt of the damage in carbon monoxid poisoning. Haldane believes that all the effects can be referred to lack of oxygen, the symptoms increasing with the saturation of the blood with carbon monoxid. He has kept mice alive on exposure to from 200 to 300 times the fatal dose of carbon monoxid in the presence of oxygen, under high pressure (from one to two atmospheres). Mosso repeated the experiment with the same result, using monkeys instead of mice. On the other hand, Lanossier says that animals die sooner and with different symptoms in an atmosphere of carbon monoxid than when asphyxiated by nitrogen. Lancereaux thinks that carbon monoxid destroys the red cells which block the vessel lumen and lead to thrombus formation. Mott believes that carbon monoxid dissolved in the blood plasma irritates the endothelium and causes necrosis and rupture. The histologic appearance of the capillaries in our series suggests that the endothelium is extremely vulnerable. The precise method of destruction of vessel walls cannot be determined without animal experimentation carefully controlled, with the use of fresh material specially prepared and stained in a more thorough manner than has yet been undertaken.

The symptoms produced by inhalation of carbon monoxid are explained by the union of this gas with the hemoglobin, for which it has an avidity estimated to be from 200 to 300 times that of oxygen, and by the vascular degeneration consequent on its presence in the circulation.

There is an initial rise in blood pressure, with slow pulse and pounding heart beat. This is followed by lowered blood pressure, rapid and small pulse, with dilatation of the peripheral blood vessels. In mild cases there is a dull headache, flashes before the eyes, dizziness, ringing in the ears, nausea and vomiting. According to Haldane, 20 per cent saturation of the blood with carbon monoxid produces these symptoms. With 50 per cent. saturation one is scarcely able to stand, and faints on slight exertion; 80 per cent. saturation is fatal. The symptoms vary with the temperament of the individual, as in alcoholic intoxication.

In severe cases there are cyanosis, difficult respiration, convulsions, paralyses, subnormal temperature, weak and intermittent pulse, and unconsciousness. Pneumonia, both lobar and lobular, follows with great frequency if the patient lives many days.

On recovery from acute poisoning, and in chronic poisoning, the end-results vary according to the previous condition of the nervous system. Existing brain lesions may be accentuated, so that carbon monoxid poisoning has been said to simulate almost every

Fig. 6.—Characteristic hyperemia and hemorrhage in the white matter of the brain in carbon monoxid poisoning.

variety of nervous disease.[12] Multiple softenings may produce the picture of insular sclerosis.[13] Paralyses, convulsive seizures, impaired memory and psychoses frequently follow. Suicidal attempts may result from insanity caused by carbon monoxid poisoning, and of course poisoning may be itself the suicidal act of one already insane.

12. McGurn, W. J.: Boston Med. and Surg. Jour., 1917, **176**, 231.
13. McGurn, W. J.: Interstate Med. Jour., 1917, **24**, 479.

Tremors, muscular spasms and pareses are accounted for by the peculiar involvement of the lenticular nucleus and the internal capsule. We cannot enter here on the disputed questions of the functions of the lenticular nucleus. Wilson[14] has recorded elaborate experiments tending to show that the corpus striatum is independent of the cerebral cortex and exerts a steadying influence on the lenticulorubrospinal projection system on innervation of the corticospinal (pyramidal) system, being concerned with the maintenance of the tone of the skeletal muscles. Several syndromes are described in neurologic literature which appear to depend on involvement of the corpus striatum, as paralysis agitans, Wilson's[15] progressive lenticular degeneration, pseudosclerosis of the lenticular nucleus,[16] Voigt's spastic pseudobulbar paralysis, Huntington's chorea, progressive athetosis,[17] and progressive atrophy of the globus pallidus.[18] Pollock[19] has recently reviewed the subject and described very similar changes to those of carbon monoxid poisoning in his case of Wilson's disease. It would perhaps be pertinent to inquire into the possibility of a chronic carbon monoxid poisoning in those diseases in which the lenticular nucleus is softened.

CONCLUSIONS

Carbon monoxid poisoning produces a characteristic lesion of the brain, namely, a bilateral ischemic necrosis of the lenticular nucleus, especially the globus pallidus. This lesion results from the vascular disturbance brought about by the presence of carbon monoxid in the circulating blood which produces a thrombosis with degeneration of the vessel walls. Anatomic peculiarities in the circulation account for the localization of the lesion. The necrosis varies from slight perivascular lesions in the globus pallidus to grossly visible softening of the entire lenticular nucleus and internal capsule, depending on the amount of gas inhaled, duration of life after poisoning, and preexisting pathologic changes in the cerebral vessels.

14. Wilson, A. S. K.: Brain, 1913-1914, **36,** 427.
15. Wilson, A. S. K.: Brain, 1912, **34,** Part 4.
16. Strumpell: Deutsch. Ztschr. f. Nervenh., December, 1915.
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When death occurs within a few hours, other evidences of carbon monoxid poisoning are available, namely, a cherry-red color of the blood, a pink hue of the brain substance, and petechial hemorrhages in the skin and various mucous and serous surfaces; further, the spectroscopic and chemical tests for carbon monoxid in the blood are positive. These evidences later disappear, and the diagnosis depends on the characteristic lesion in the lenticular nucleus.

Spontaneous hemorrhages in the leptomeninges and punctiform hemorrhages throughout the white matter of the brain are part of the profound vascular disturbances in carbon monoxid poisoning.

Nerve cells possess a high degree of tolerance to the poisonous action of carbon monoxid.

Edema and hyperemia of the brain and internal hydrocephalus are frequent appearances in carbon monoxid poisoning.

Cerebral arteriosclerosis cannot be attributed to carbon monoxid, but it probably increases the vulnerability of the individual to small amounts of the gas.

Carbon monoxid poisoning as a cause for the subsequent development of nervous and mental disease as well as for deaths which may not be explained easily receives emphasis from this study. The occupation and other conditions of living as well as careful gross and microscopic examination of the brain are of importance in demonstrating such a causal relationship.

REPORT OF CASES

The 32 cases studied are summarized as follows:

CASE 1.—Woman, aged 55, survived three days.

Clinical Diagnosis: Cerebral hemorrhage.

Service of Dr. Patton.

Clinical Symptoms and Signs: Coma; deviation of the eyes to the right; flaccidity of arms and legs; spinal fluid under pressure, the latter portion mixed with blood.

Anatomic Diagnosis: Carbonylemia; infarct of the spleen; hypostatic hyperemia of the lungs; cholelithiasis; chronic indurative cholelithiasis; fatty infiltration of the pancreas; anemia; marked senile sclerosis of the abdominal aorta; sclerosis of the anterior mitral leaflet; atrophy of the liver and kidneys; postclimacteric atrophy of the genital organs; cavernous angioma of the under surface of the liver.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; marked bilateral softening of

the putamen, globus pallidus and internal capsule; edema of the brain; internal hydrocephalus; fibrous ependymitis; multiple cysts in the choroid plexus; marked sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Cortex and Leptomeninges: Edema, hyperemia, multiple spherical bodies (lipoid?) along the granular layer of the cortex and under the leptomeninges, punctiform hemorrhages in the leptomeninges.

Lenticular Nucleus: Marked hyperemia, thromboses and degenerated vessel walls, extensive necrosis, capillary hemorrhages.

Internal Capsule: Same changes but less pronounced.

Caudate Nucleus: Pressure atrophy and necrosis, multiple spherical bodies around the vessels and lymph spaces.

Corona Radiata, Pons and Brain Stem: Hyperemia.

Cerebellum: No changes.

Remarks: Patient was overcome by gas in her room; no other history available.

Case 2.—Man, aged 22, survived three days.

Clinical Diagnosis: Gas poisoning.

Service of Dr. Carr.

Clinical Symptoms and Signs: Flushed face; eyes closed; jaws tight; flaccid extremities; roughened breath sounds.

Anatomic Diagnosis: Petechial hemorrhages of the lining of the renal pelves, beneath peritoneum in front of the urinary bladder, in the lining of the urinary bladder and beneath the pleura of left lung; acute emphysema of the left lung; fluid blood in superior vena cava; cloudy swelling of the kidneys and myocardium; fatty changes in lining of the aorta and the coronary arteries; calcification of mesenteric lymph glands; slight sclerosis of the front mitral leaflet; scoliosis.

Macroscopic Examination of the Brain: Disseminated atrophy of the cerebral cortex; hyperemia of the brain and leptomeninges; petechial hemorrhages and edema of the leptomeninges; sclerosis of the cerebral arteries.

Microscopic Examination of the brain:

Cortex and Leptomeninges: Edema and capillary hemorrhages; thrombosis; degenerated vessel walls.

Corona Radiata: The same.

Lenticular Nucleus: Sclerosis of vessel walls; thrombosis; periarteritis; edema; pyknosis of glial nuclei and slight necrosis.

Remarks: The patient was found in a gas-filled room; chemical test for carbon monoxid in the blood was positive.

Case 3.—Woman, aged 60, survived three days.

Clinical Diagnosis: Carbon monoxid poisoning.

Service of Dr. Tice.

Clinical Symptoms and signs: Coma; unequal pupils; slight icterus of the conjunctivae; rapid and labored respiration; rapid and irregular pulse; moist râles; temperature just before death was 104.2.

Anatomic Diagnosis: Multiple hemorrhages of the visceral pleura; cloudy swelling of the kidneys; marked hypostatic hyperemia and bronchopneumonia of the lungs; marked hypostatic hyperemia of the pharyngeal mucosa; chronic tonsillitis; hyperemia of the lining of the stomach; slight senile sclerosis of the abdominal aorta; marked sclerosis of the anterior mitral leaflet; left pyosalpingitis; cholelithiasis; fibromyomas of the uterus.

Macroscopic Examination of the brain: Hyperemia of the brain and leptomeninges; petechial hemorrhages in the leptomeninges; syphilitic scars in the leptomeninges; multiple punctiform hemorrhages in the cerebrum and cerebellum; bilateral hemorrhagic softening in the corpus striatum, cerebrum, cerebellum, pons and medulla; marked internal hydrocephalus; saccular aneurysm of the basilar and right middle cerebral artery; marked sclerosis of the cerebral arteries.

Microscopic examination of the Brain:

Lenticular Nucleus and Internal Capsule: Very marked necrosis, edema, thrombosis, arteriosclerosis, periarteritis.

Remarks: Patient had a dizzy spell while cooking and fell, disconnecting the gas tube; she was found unconscious by the neighbors. She had suffered a stroke of paralysis when a child, after which her face was drawn to one side.

Case 4.—Man, aged 60, survived eleven days.

Clinical Diagnosis: Cerebral hemorrhage.

Service of Dr. Hassin.

Clinical Symptoms and Signs: Coma; immobility of the right side of the mouth; paralysis of the right upper extremity; unequal pupils, not reacting to light; odor of gas to breath; cerebrospinal fluid bloody.

Anatomic Diagnosis: Spontaneous cerebral hemorrhage; disseminated hemorrhages in the leptomeninges; marked hyperemia of the brain and leptomeninges; marked internal hydrocephalus; marked sclerosis of the cerebral arteries; disseminated atrophy of the cerebral cortex; parietal thrombus in the basilar artery.

Microscopic Examination of the Brain:

Leptomeninges: Fibrous thickening of the pia; multiple small hemorrhages; many thrombosed vessels; marked arteriosclerosis.

Cortex: Nerve cells normal; multiple hemorrhages in occipital cortex.

Internal Capsule: Ischemic necrosis; sclerosis and thrombosis of the vessels.

Lenticular Nucleus: The same.

Remarks: Patient was overcome by gas, April 30, and was taken to the hospital, where he remained until May 7. May 9

he fell down stairs and was found unconscious. There had been no paralysis on his first admission to the hospital.

Case 5.—Man, aged 40, survived twenty-four hours.

Clinical Diagnosis: Gas poisoning.

Service of Dr. Williamson.

Clinical Symptoms and Signs: Gas odor to breath; coma; temperature 102, pulse 140, respirations 36; stertorous breathing; all reflexes absent; coarse râles over both lungs; hemoglobin, 115 per cent.; leukocytes, 19,000; temperature rose to 106 before death.

Anatomic Diagnosis: Petechial hemorrhages in the skin and gastric mucosa; cloudy swelling of the kidneys; fatty changes in the kidneys; fatty changes in the liver; slight epicardial fatty infiltration; hypostatic hyperemia and edema of the lungs; hypostatic hyperemia of the bowels and trachea; slight hyperplasia of the spleen and the intestinal lymphoid tissue; emphysema of the lungs; hypertrophy of right and left ventricle of heart; mucopurulent bronchitis; sclerosis of the aorta and iliac bifurcation; slight sclerosis of the front mitral leaflet; scars in the right inguinal region.

Macroscopic Examination of the Brain: Disseminated atrophy of the cerebral cortex; marked hyperemia of the brain and leptomeninges; syphilitic fibrous leptomeninges; edema of the brain; internal hydrocephalus; marked sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Leptomeninges: Fibrous thickening; edema; hyperemia; marked sclerosis of the vessels.

Cortex: Moderate edema.

Lenticular Nucleus and Internal Capsule: Edema; hyperemia; thrombosis; degeneration of the vessel walls; arteriosclerosis.

Medulla Oblongata: The same.

Remarks: The patient was found in a gas-filled room in coma; he had been despondent and had committed suicide. Wetzel test for carbon monoxid in the blood was negative.

Case 6.—Man, aged 74, survived one month.

Clinical Diagnosis: Senile dementia.

Service of Dr. Miller.

Clinical Symptoms and Signs: Short stertorous breath; cough; moist râles over back of lungs; hyperresonance over front; blood pressure 120-60; leukocytes 14,000; pulse 88, respiration 24, temperature 99, rising to 102 two days before death.

Macroscopic Examination of the Brain: Contusion hemorrhage and hemorrhagic softening of occipital cortex; bilateral softening of the lenticular nucleus; marked sclerosis of the cerebral arteries; fibrous ependymitis.

Microscopic Examination of the Brain:

Leptomeninges and Cortex: Hyperemia; edema.

Corpus Callosum: Edema.

Lenticular Nucleus and Internal Capsule: Ischemic necrosis; thrombosis; distended and degenerated vessels; punctiform hemorrhages.

Corona Radiata: Marked thrombosis; distended and degenerated vessels.

Brain Stem and Pons: Hyperemia and edema.

Remarks: Patient on returning to his home found the house full of gas; he entered and raised the windows; he remembered nothing after this until he became conscious after two days in the hospital. When he had sufficiently recovered to leave the hospital, he was intent on suicide and was sent to the psychopathic ward, where he died.

Case 7.—Man, aged 37, survived eleven days.

Clinical Diagnosis: Gas poisoning; lobar pneumonia.

Service of Dr. Capps.

Clinical Diagnosis, Symptoms and Signs: Gas odor to breath; unconscious; pulse 88, respiration 28, temperature 97.

Anatomic Diagnosis: Bronchopneumonia with gangrene of the entire right lung.

Macroscopic Examination of the Brain: Disseminated atrophy of the cerebral cortex; hyperemia of the brain and leptomeninges; moderate internal hydrocephalus; edema of the brain; sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Leptomeninges and Cortex: Hyperemia; edema; thrombosis.

Corpus Callosum: Slight edema and hyperemia.

Centrum Semiovale: Slight thrombosis; edema.

Lenticular Nucleus and Internal Capsule: Slight necrosis; slight thrombosis; arteriosclerosis.

Medulla Oblongata: Edema; hyperemia; excessive pigment granules in the nerve cells.

Wassermann test positive.

Case 8.—Man, aged 32, survived seven days.

Clinical Diagnosis: Lobar pnuemonia, chronic alcoholism, delirium tremens.

Service of Dr. Beifeld.

Clinical Symptoms and Signs: On entrance the blood pressure was 100, the temperature 101, pulse 116, respirations 36, and he had an area of dulness over the right lung. White blood corpuscles 18,000; patient was somewhat irrational and had marked tremor; next day the temperature rose, pulse became rapid and weak, respiration loud and labored. This condition progressed until death.

Anatomic Diagnosis: Lobar pneumonia; hyperemia of the bulbar conjunctivae; hyperplasia of the spleen; fatty liver; hypostatic hyperemia and edema of the lungs; left fibrous pleuritis; chronic catarrhal otitis media; syphilitic scars of the legs.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; internal and external hydrocephalus; edema of the brain and leptomeninges; multiple punctiform hemorrhages in the brain substance; diffuse intrameningeal hemorrhage; slight sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Leptomeninges: Hyperemia; thrombosis; degeneration of the vessel walls; capillary hemorrhages; edema; arteriosclerosis; fibrous thickening of the arachnoid.

Cortex: Spherical bodies surrounding the vessels under the pia; hyperemic and collapsed and degenerated vessels; thrombosis.

Lenticular Nucleus: Hyperemic and collapsed vessels; thrombosis; slight degeneration of the vessel walls; edema; capillary hemorrhage surrounded by small regions of necrosis.

Corona Radiata: Capillary hemorrhages; multiple thrombi containing fibrin and polymorphs; empty and collapsed vessels.

Corpus Callosum: Hyperemia; thrombosis; edema; pressure necrosis; multiple spherical bodies.

Medulla: Thrombosed vessels containing multiple polymorphs, fibrin and spherical bodies; perivascular necrosis surrounding empty vessels, especially at floor of fourth ventricle.

Remarks: Had been take to Cook County Hospital from his home, where he had been sick seven days. Patient gave history of heavy drinknig for some time and fever and chills the last week.

Case 9.—Woman, aged 78, survived ten days.

Clinical Diagnosis: Gas poisoning, asphyxia.

Service of Dr. Butler.

Clinical Symptoms and Signs: Coma, cyanosis; stertorous breathing; small pupils; increased reflexes; positive Babinski.

Anatomic Diagnosis: Large epicardial hemorrhage of the right auricle; petechial hemorrhage of the lining of the parietal pleura; marked epicardial fatty infiltration of front of heart; edema of subepicardial adipose tissue; nearly empty right heart and pulmonary artery; moderate general anemia; senile arteriosclerosis; atheromatous ulcers of the abdominal aorta; slight fibrous pachymeningitis; general senile atrophy of the viscera; calcified nodule in the liver; large fibrous epicardial patches; senile emphysema of the lungs; marked hypostatic hyperemia of the lungs.

Macroscopic Examination of the Brain: General senile atrophy of the cerebral cortex; hyperemia of the brain and leptomeninges; edema of the brain and leptomeninges; internal hydrocephalus; cystic degeneration of the choroid plexus; marked sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Cortex and Leptomeninges: Extreme sclerosis of the vessel walls; hyperemia; edema.

Corona Radiata: Edema; hyperemia; sclerosed vessels; enormous masses of spherical bodies.

Lenticular Nucleus: Extreme arteriosclerosis; some calcification; edema; slight necrosis; vacuolization.

Corpus Callosum: Hyperemia; edema; multiple spherical bodies around ependyma and in septum pellucidum.

Remarks: Was well the day before; gas leaking in the room.

Case 10.—Man, aged 50, survived six days.

Clinical Diagnosis: Gas poisoning.

Service of Dr. Irons.

Clinical Symptoms and signs: Comatose; could not be aroused with pulmotor; skin cherry red; imperceptible radial pulse; rapid, regular weak heart; slight edema of extremities; positive bilateral Babinski; respiration irregular in rate and depth; temperature from 98 to 101 and at one time as high as 105.

Anatomic Diagnosis: Cherry-red blood; petechial hemorrhage in the greater antrum of the stomach and in the renal pelves; disappearing subpial hemorrhage; marked hyperemia of the lining of the pharynx, larynx and trachea; putrid and suffocative bronchopneumonia; marked edema and hypostatic hyperemia of the lungs; fatty changes of the liver; slight fatty changes of the aorta; thrombosis of the right vertebral artery; lime deposits in the wall of the left ophthalmic artery; healed infarct of the spleen; fibrous epicardial patches; right fibrous pleuritis; subepicardial and periarterial fibrous patches (syphilis); multiple subscapular gummatous scars of the liver.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; fibrous perivascular leptomeningitis; edema of the brain and leptomeninges; traumatic hemorrhage in the brain and leptomeninges; localized hemorrhage softening of the brain; sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Lenticular Nucleus and Internal Capsule: Necrosis; degeneration of vessel walls; multiple minute hemorrhages; multiple spherical bodies surrounding the degenerated vessels and spreading in the necrosed regions; marked sclerosis of the vessel walls; marked edema; some swollen nerve cells with excentric nucleus.

Corona Radiata: Marked edema; multiple hemorrhages; degenerated vessel walls; marked arteriosclerosis.

Remarks: Found in bathroom with gas tube in his mouth and gas jet open.

Case 11.—Man, aged 25, survived a few hours.

Clinical Diagnosis: None given.

Service of Dr. Morf.

Anatomic Diagnosis: Traumatic fracture of the cranial bones; the superior maxilla, the left femur, and tibia and fibula and os calcis; bruised brain; subdural hemorrhage;

cherry-red blood (carbonylemia?); subepicardial petechial hemorrhages; multiple external bruises and lacerated wounds; lessened yellow material in the suprarenal cortices; fatty liver; cloudy swelling of the kidneys and myocardium; hyperplasia of the spleen; right fibrous pleuritis; hypostatic hyperemia of the lungs.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; edema of the brain.

Microscopic Examination of the Brain:

Lenticular Nucleus and Internal Capsule: Necrosis, especially of globus pallidus; thrombosis; perivascular agglomeration of spherical bodies; degenerated vessel walls; edema.

Corpus Callosum: Hyperemia; sclerosis of vessels; degeneration of vessels; thrombosis of subependymal vessels; edema; some vessels hyperemic.

Remarks: Was brought to the hospital with multiple injuries from an explosion; patient was very ill and no history was obtainable; during all the time he was semiconscious and very restless. Wassermann was negative.

Case 12.—Man, aged 64, survived twenty-four hours.

Clinical Diagnosis: Injury due to explosion of gas.

Service of Dr. Andrews.

Clinical Symptoms and Signs: Semicoma, restlessness.

Anatomic Diagnosis: Traumatic fracture of the sternum; right, first, third, fourth, fifth and seventh ribs and left third to eighth ribs (fat embolism?); extensive hemorrhage about fractures; hemorrhage in tissue of scalp; blood-stained cerebrospinal fluid; cloudy swelling of the kidneys; hyperplasia of the spleen; sclerosis of anterior mitral leaflet; slight fatty liver; external fibrous pachymeningitis; left apical fibrous pleuritis; caseous, nodular and encapsulated tuberculosis of the apex of the left lung; fibrous interstitial prostatitis; hypostatic hyperemia of the lungs; edema of the lungs.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; marked internal hydrocephalus; punctiform hemorrhages (?) in the nucleus dentatus; bilateral softening of the lenticular nucleus (very slight); fibrous perivascular leptomeningitis; sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Leptomeninges: Enormous fibrous thickenings; arteriosclerosis.

Cortex: Vessels collapsed; edema; cell atrophy.

Lenticular Nucleus: Hyperemia; some vessels collapsed; perivascular necrosis in internal capsule and globus pallidus, surrounded by clusters of spherical bodies; edema; thrombosis; slight necrosis in putamen.

Remarks: Explosion injury; no history; Wassermann negative.

Case 13.—Man, aged 40, survived twenty-four hours.

Clinical Diagnosis: Gas poisoning; chronic nephritis; encephalitis.

Service of Dr. Kerr.

Clinical Symptoms and Signs: In coma, respiration labored, stertorous, 30 per minute, the sputum being blown from mouth and nostrils; face cyanotic; cold extremities; gas odor to breath; pupils small, irregular, not responding to light; blood pressure, systolic 136, dyastolic 82. R. B. C., 4,800,000; W. B. C., 17,000; polymorphs, 70 per cent. small, large 1 per cent.; temperature 104.6 to 108 before death.

Anatomic Diagnosis: Hyperemia; clot-distended right heart; hyperplasia of the spleen; cloudy swelling of the myocardium; markedly urine-distended bladder; first degree burns of the right arm and left hand; marked hypostatic hyperemia of the lungs; persistent lymphoid tissue in the thymus; hyperplasia of the lymphoid tissue at the back of the tongue, of the upper and deep cervical lymph glands and of the biliary lymph glands (status lymphaticus?); slight apical, nodular calcified and fibrous tuberculosis of the lungs; slight sclerosis of the anterior mitral leaflet; brown pigmented scars of the left leg.

Macroscopic Examination of the Brain: Marked hyperemia of the brain and leptomeninges; extensive hemorrhage in the leptomeninges; marked internal hydrocephalus; bilateral softening of the lenticular nucleus; moderate sclerosis of the cerebral arteries; fibrous ependymitis; edema of the brain.

Microscopic Examination of the Brain:

Lenticular Nucleus and Internal Capsule: Marked necrosis; vessels containing many polymorphonuclear leukocytes; edema; hyaline thrombi.

Corona Radiata: Necrosis; marked edema; hyaline thrombi; marked hyperemia.

Cortex: Edema.

Remarks: Was found in his room unconscious; consciousness did not return. Wetzel test for carbon monoxid positive. Wassermann negative.

Case 14.—Man, aged 26, survived forty-eight hours.

Clinical Diagnosis: Gas poisoning.

Service of Dr. Hamburger.

Clinical Symptoms and Signs: Semicomatose; pulse grew weaker, respiration shallow.

Anatomic Diagnosis: Edema of the brain; marked hyperemia of the leptomeninges; petechial hemorrhages in the pleura, pericardium, renal pelves and ureters; venesection wounds; hypostatic bronchopneumonia of the lower right lung; marked hyperemia and edema of the lungs; acute catarrhal conjunctivitis; petechial hemorrhages of the gastric mucosa; hyperplasia of the spleen; sclerosis of the anterior mitral leaflet and lining of the aorta; passive hyperemia of the liver, the lining of the bowels and suprarenals; cloudy swelling of the kidneys and pancreas; fatty liver; hyperplasia of

the biliary and tracheobronchial lymph glands; fibrous pleuritis; chronic follicular tonsillitis.

Macroscopic Examination of the Brain: Marked hyperemia of the brain and leptomeninges; punctiform hemorrhages in the white matter of the brain; edema of the brain; internal hydrocephalus.

Microscopic Examination of the Brain:

Lenticular Nucleus: Marked edema; hyperemia; pyknoses; thrombosis.

Corona Radiata: Edema; hyperemia; multiple capillary hemorrhages; fibrin shreds in the vessels; degenerated arterioles, with faintly staining granular contents.

Remarks: Found overcome by gas in his room.

Case 15.—Man, aged 46, survived six weeks.

Clinical Diagnosis: Gas poisoning; lobar pneumonia; tuberculous bronchopneumonia.

Service of Dr. Post.

Clinical Symptoms and Signs: Gas odor to breath, cyanotic but conscious; blood pressure 90; pulse 120; rectal temperature 100.2. The last few days the temperature rose to 104 and patient became weaker.

Anatomic Diagnosis: Extensive caseous bronchopneumonia; ulcerative tuberculosis of the lungs; marked edema and phlegmonous inflammation of the nontuberculous lung tissue; bilateral fibrinopurulent pleuritis; encapsulated abscess of the pleural surface of the right leaf of the diaphragm; caseous and nodular tuberculosis of the tracheobroncheal lymph glands; purulent bronchitis and tracheitis; acute emaciation; marked anemia; fibrous pleuritis; hyperplasia of the spleen; biliary, periaortic and tracheobroncheal lymph glands; cloudy swelling of the kidneys and liver; fatty liver; petechial hemorrhages of the lining of the stomach, the duodenum and the skin of the cheek; senile arteriosclerosis of the root of the aorta and coronary arteries; slight hypertrophy of the left ventricle of the heart; slight ascites; edema of the leptomeninges; passive hyperemia of the bowels.

Macroscopic Examination of the Brain: Partial anemia and hyperemia of the brain and leptomeninges; punctiform hemorrhages in the cerebrum, cerebellum and brain stem; edema of the brain and leptomeninges; moderate internal hydrocephalus; syphilitic scars (?) in the leptomeninges; marked sclerosis of the cerebral arteries; disseminated atrophy of the cerebral cortex; cysts in the choroid plexus.

Microscopic Examination of the Brain:

Lenticular Nucleus and Internal Capsule: Ischemic necrosis; thrombosis; edema; degeneration of the vessel walls; multiple spherical bodies.

Corona Radiata: Some hyperemic and many empty and collapsed vessels; slight necrotic changes; edema.

Brain Stem and Pons: Hyperemia; thrombosis; punctiform hemorrhages; degenerated vessels; edema.

Leptomeninges: Fibrosis; thickened vessels; edema; periarteritis and endarteritis.

Cortex: Thickened vessels; some congested vessels and others collapsed and empty; edema; multiple spherical bodies under the pia, extending into the cortex.

Caudate Nucleus: Edema.

Remarks: Was found in the basement with closed door and gas jet open.

Case 16.—Man, aged 34, survived three days.

Clinical Diagnosis: Gas poisoning.

Service of Dr. Loeb.

Clinical Symptoms and Signs: Gas odor to breath; respiration stertorous, 28 per minute; feeble; rapid pulse, 90 per minute; involuntary evacuations; during examination comatous. Blood pressure, 121-82; carbon monoxid test positive with sodium hydroxid.

Anatomic Diagnosis: Cherry-red blood (carbonylemia); hyperemia and edema of the leptomeninges; hyperemia of the trachea and bronchi; bronchopneumonia; cloudy swelling of the kidneys; petechial hemorrhages in the lining of the stomach.

Macroscopic Examination of the Brain: Disseminated atrophy of the cerebral cortex; marked hyperemia of the brain and leptomeninges; disseminated hemorrhages in the leptomeninges; multiple punctiform hemorrhages in the brain substance; moderate internal hydrocephalus; moderate sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Lenticular Nucleus: Edema; arteriosclerosis; slight perivascular necrosis; degeneration of the vessel walls; pigment granules covering the vessel walls and cells.

Corona Radiata: Hyperemia; thrombosis; edema; capillary hemorrhages.

Remarks: Found in gas-filled room; Wassermann negative.

Case 17.—Man, aged 45, survived three days.

Clinical Diagnosis: Gas poisoning and bronchopneumonia.

Service of Dr. Vaughan.

Clinical Symptoms and Signs: Deep coma; respiration stertorous and rapid; eyeballs rolled from side to side; face cyanotic; pupils contracted and reacted very slowly to light; the conjunctival vessels were dilated; numerous râles over both lungs; blood pressure, 115 systolic, 80 dyastolic; blood transfusion was made, temperature rose to 103. Next day pulse was fair and respiration rapid. One thousand c.c. salt solution were injected intravenously for two days; just before death temperature was 105, pulse 104 and respiration 60.

Anatomic Diagnosis: Symmetric hemorrhages of the brain; hypostatic edema of the lungs; hyperemia of the lining of the trachea, bronchi, larynx and pharynx; slight cloudy swelling of the kidneys; petechial hemorrhages in the stomach and

duodenum; chronic, nodular, miliary, caseous tuberculosis of the right lung; chronic encapsulated tuberculosis of the lungs; acute emaciation; passive hyperemia of the liver; petechial hemorrhages in the pelvis of the left kidney; fatty changes in the lining of the descending aorta and aortic root; small gummatous scars of the liver; fibrous obliteration of the left pleural cavity.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; traumatic and punctiform hemorrhages in the brain and leptomeninges; disseminated softening of the brain substance; marked sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Leptomeninges and Cortex: Large hemorrhages; sclerosed vessels; thrombosis and edema.

Corpus Striatum: Multiple thrombi; hemorrhages; edema; extreme necrosis; sclerosis of the vessels.

Corona Radiata: Hyperemia; punctiform hemorrhages; edema.

Cerebellum: Punctiform hemorrhages; thrombosis; sclerosis of the vessels.

Pons and Medulla: Capillary hemorrhages; thrombosis; sclerosis of the vessels; calcification of the vessels.

Remarks: Patient was overcome by gas and had been ill thirty-six hours before entering the hospital. Test for carbon monoxid negatuve. Wassermann negative.

Case 18.—Man, aged 43, survived thirty-six hours.

Clinical Diagnosis: Gas poisoning.

Service of Dr. Bloch.

Clinical Symptoms and Signs: Stertorous breathing; cyanotic; rapid pulse; pupils dilated, equal and do not react to light or accommodation; cherry-red color of ears; bronchovesicular breathing; moist râles over the apices of the lungs and the large bronchi; hyperresonance over the anterior and posterior chest walls; flaccid extremities; temperature 103.4; pulse 112, respiration 43; temperature rose to 106.8 before death.

Anatomic Diagnosis: Right and left lobar pneumonia; hypostatic bronchopneumonia; bilateral fibrinous pleuritis; hyperplasia of the tracheobronchial lymph glands; cloudy swelling of the myocardium, kidneys and liver; hyperemia of the renal pelves; hypertrophy of the heart; bilateral hydrocele.

Macroscopic Examination of the brain: Hyperemia of the brain and leptomeninges; edema of the brain; extreme bilateral softening of the lenticular nucleus, especially the globus pallidus in the posterior portion; multiple punctiform hemorrhages in the corpus callosum and in the white matter; pink discoloration of the white substance of the brain; marked sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Corpus Callosum: Hyperemia; edema; multiple capillary hemorrhages and degenerated vessel walls.

Remarks: Found unconscious in bed; had been sick twenty-four hours.

Case 19.—Woman, aged 55, survived five days.

Clinical Diagnosis: Gas poisoning, cerebrospinal syphilis, lobar pneumonia, cystic ovary.

Service of Dr. Butler.

Anatomic Diagnosis: Unequal dilated pupils; acute emaciation; marked sclerosis of the aorta; fibrous external and internal pachymeningitis; moderate fatty infiltration of the front wall of the heart; slight fatty changes of the kidneys and the liver; senile atrophy of the liver and spleen; brown atrophy of the heart; fibrous pelvic peritonitis; sclerosis of the anterior mitral leaflet.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; syphilitic leptomeningitis; slight intrameningeal hemorrhage; bilateral softening of the lenticular nucleus; moderate internal hydrocephalus; marked sclerosis of the cerebral arteries; patent fifth ventricle.

Microscopic Examination of the Brain:

Leptomeninges: Endarteritis and periarteritis; punctiform hemorrhages; fibrous thickening.

Cortex: Arteriosclerosis.

Lenticular Nucleus: Thrombosis; degeneration of the vessels; ischemic necrosis.

Caudate Nucleus: Very slight necrotic changes.

Corona Radiata: Degenerated vessels; punctiform hemorrhages; focal necrosis.

Brain Stem: Marked periarteritis and endarteritis; sclerosis of the vessels; one dissecting aneurysm; ischemic necrosis.

Remarks: Was found in her room with gas jet turned on. Wassermann positive.

Case 20.—Man, aged 38, survived two days.

Clinical Diagnosis: Multiple lacerations of hands and face.

Service of Dr. Davison.

Clinical Symptoms and Signs: Delirium.

Anatomic Diagnosis: Traumatic comminuted fractures of the skull; traumatic fracture of the sixth and seventh ribs; contusion of the brain; traumatic hemorrhage of the brain, the chest wall and the right temporal muscle; small extradural traumatic hemorrhage; surgically repaired laceration of the scalp; slight fatty liver; hyperplasia of the spleen and the intestinal lymphoid tissue; healed calcified tuberculosis of the tracheobronchial lymph glands; hyperemia of the trachea; slight sclerosis of the coronary arteries; atherosclerosis of the aorta; right fibrous pleuritis; emphysema of the lungs; copper color pigmentation of the skin of the legs (syphilitic?).

Macroscopic Examination of the Brain: Extensive contusion hemorrhage in the leptomeninges; edema of the brain; multiple punctiform hemorrhages in the cerebrum and cerebellum; circumscribed bilateral softening of the corpus striatum; large cysts in the choroid plexus of the fourth ventricle; marked sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Lenticular Nucleus and Internal Capsule: Marked necrosis; sclerosis of the arteries; edema.

Remarks: Found in a building wrecked by an explosion.

Case 21.—Man, aged 25, survived thirty-six hours.

Clinical Diagnosis: Broken neck.

Service of Dr. Burlingame.

Clinical Symptoms and Signs: Coma; cyanosis; stertorous respiration; pupils equal and small; reflexes sluggish; gas odor to breath; pulse 80, respiration 18, temperature 94.5; leukocytosis 52,000; vomited undigested food; temperature rose to 105 and death followed.

Anatomic Diagnosis: Hypostatic hyperemia of the lungs; edema of the lungs; slight fibrous pleuritis; scars of the legs.

Macroscopic Examination of the Brain: Edema of the brain and the leptomeninges; hyperemia of the brain and leptomeninges; punctiform hemorrhages in the brain substance; bilateral softening of the lenticular nucleus.

Microscopic Examination of the Brain:

Lenticular Nucleus: Very marked necrosis; arteriosclerosis; thrombosis; edema.

Corona Radiata: Punctiform hemorrhages; recent thrombi; edema; sclerosis of the arteries.

Internal Capsule: The same.

Pons: Sclerosis of the arteries; thrombosis; edema.

Case 22.—Woman, aged 40, survived twelve hours.

Clinical Diagnosis: Suffocation.

Service of Dr. Patton.

Anatomic Diagnosis: Carbonylemia; petechial hemhorrhages of the skin; acute fatty changes in the myocardium; edema of the lungs; hydrothorax; hydropericardium; passive hyperemia of the liver, bowels and renal pelves; fatty changes of the lining of the aorta; hyperplasia of the spleen; petechial hemorrhages of the lining of the stomach and duodenum; cloudy swelling of the kidneys; hyperplasia of the tracheobronchial lymph glands; biliary periaortic and solitary lymph glands of the bowels; pregnant uterus; corpus luteum of pregnancy in the left ovary; lactating breasts; fibrous pleuritis; fibroid of the uterus; slight internal and external pachymeningitis; scoliosis; compensatory emphysema of the lungs; fibrous epicardial patch; syphilitic scars of the legs.

Macroscopic Examination of the Brain: Hyperemia and edema of the leptomeninges; petechial hemorrhages in the

leptomeninges; edema of the brain; slight sclerosis of the cerebral arteries; bright pink coloration of the brain tissue.

Microscopic Examination of the Brain:

Lenticular Nucleus: Marked edema; arteriosclerosis; degenerated and empty vessels; coagulation thrombosis; hydrops of nerve cells; necrosis of the large neurons in the internal capsule.

Corona Radiata: Very marked edema; degenerated vessel walls; minute hemorrhages.

Leptomeninges: Edema; slight sclerosis of the vessels; punctiform hemorrhages; endarteritis and periarteritis.

Remarks: Patient was well in the morning; during the day a stove exploded and she suffocated in a burning building. Carbon monoxid test positive.

The following reports are of patients that were brought to the Cook County Hospital either dead or who died within a few hours after admission:

Case 23.—Man, aged 35, survived three days.

Clinical Symptoms and signs: Unconsciousness; cyanosis; vomiting; rigid jaws; pulse 110, respiration 50, temperature 106 before death; patient did not regain consciousness.

Anatomic Diagnosis (only the brain was examined): Marked hyperemia of the brain and leptomeninges; edema of the brain; bilateral softening of the lenticular nucleus; disseminated atrophy of the cerebral cortex; slight sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Cortex and Leptomeninges: Hyperemia and edema.

Corpus Callosum: Edema.

Lenticular Nucleus and Internal Capsule: Ischemic necrosis; thrombosis; punctiform hemorrhages; degenerated vessel walls.

Corona Radiata: Marked thrombosis; distended and degenerated vessels.

Brain Stem and Pons: Hyperemia.

Remarks: Patient was found in his room with the gas jet open; Wetzel test for carbon monoxid negative; Wassermann test negative.

Case 24.—Man, aged 27, found dead.

Anatomic Diagnosis: Carbonylemia; petechial hemorrhages of the skin; hyperemia and edema of the lungs; hyperplasia of the spleen and of the thyroid gland; scars of the liver and the penis.

Macroscopic Examination of the Brain: Edema of the brain; slight hyperemia of the leptomeninges; slight sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Cortex: Edema; slight degeneration of the vessel walls.

Lenticular Nucleus and Internal Capsule: Slight perivascular necrosis; slight thrombosis; edema.

Pons and Medulla: Very marked edema.

Cerebellum (dentate nucleus): Very marked edema.

Remarks: Patient was found in a gas-filled room; his sister believed that he had committed suicide. Wetzel test for carbon monoxid positive.

Case 25.—Man, aged 40, survived a few hours.

Anatomic diagnosis: Traumatic fracture of the left second to sixth ribs, inclusive, and of the sternum; fresh hemorrhages about the fractures; cherry-red blood; bruise of the retroperitoneal tissue; external bruises and lacerated wounds; fatty liver; fatty changes in the aorta; sclerosis of the coronary arteries; left fibrous pleuritis.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; edema of the brain; slight sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Lenticular Nucleus: Edema; hyalin thrombi; sclerosed arteries; pyknoses; capillary hemorrhages.

Corona Radiata: Slight periarteritis; edema; thrombosis; capillary hemorrhages.

Cortex: Slight edema; arteriosclerosis.

Leptomeninges: Edema; sclerosis of the arteries; minute hemorrhages; periarteritis.

Remarks: Killed by an explosion.

Case 26.—Man, aged 19, survived a few hours.

Anatomic Diagnosis: Multiple abrasions and lacerated wounds; minute fresh petechial hemorrhages in the pleura and thymus; cherry-red blood; bruised scalp; fatty changes in the aorta; moderate hyperplasia of the spleen, of cervical lymph glands, mucosa of the stomach and also the solitary lymph nodes of the ileum; persistent lymphoid tissue in the thymus; passive hyperemia of the kidneys; hypostatic hyperemia of the lining of the bowels.

Macroscopic Examination of the Brain: Disseminated atrophy of the cerebral cortex; hyperemia of the brain and the leptomeninges; edema of the brain; slight internal hydrocephalus; pink coloration of the brain substance.

Microscopic Examination of the Brain:

Lenticular Nucleus and Internal Capsule: Necrosis; edema; thrombosis; degeneration of the vessel walls.

Cortex and Corona Radiata: Hemorrhages; degeneration of the vessel walls; edema.

Remarks: Injured by explosion; died within a few hours in the receiving room of the Cook County Hospital.

Case 27.—Man, aged 40, survived twenty hours.

Clinical Symptoms and Signs: Stupor, coma and death.

Anatomic Diagnosis: Traumatic fracture of the bones of pelvis and bones of both legs; traumatic hemorrhage of the bladder wall, scalp, mediastinal tissue and about lumbar and sacral vertebrae; petechial hemorrhage in the lining of the

urinary bladder; blood in tunica vaginalis testis; acute general anemia; cloudy swelling of the kidneys; hyperplasia of the spleen; fatty liver; slight sclerosis of the anterior mitral leaflet.

Macroscopic Examination of the Brain: Partial anemia and hyperemia of the leptomeninges; edema of the brain and leptomeninges; anemia of the brain; fibrous leptomeningitis; fibrous ependymitis; perivascular atrophy of the cortex; moderate sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Lenticular Nucleus: Perivascular necrosis; thrombosis; edema; arteriosclerosis; multiple spherical bodies about the vessels and in the degenerated regions.

Corona Radiata: Hyperemia; necrosis; edema; thrombosis; degenerated vessels.

Caudate Nucleus and Corpus Callosum: Edema.

Pons: Hyperemia; arteriosclerosis; edema; capillary hemorrhages.

Leptomeninges: Fibrous thickening; edema.

Cortex: Edema.

Remarks: Entered the hospital in a semicomatose condition from injuries received in an explosion.

Case 28.—Woman, aged 65, survived a few hours.

Anatomic Diagnosis: Senile sclerosis of the aorta (syphilitic?); sclerosis of the arch of the aorta; slight stenosis of the aorta arch; hypertrophy of the left ventricle; dilated superficial veins of the extremities; slight arteriosclerotic atrophy of the kidneys; slight fatty changes of the liver; fibrous external pachymeningitis.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; spontaneous hemorrhages in the leptomeninges; external and internal hydrocephalus; slight sclerosis of the cerebral arteries; multiple cysts in the choroid plexus; general atrophy of the cerebral cortex.

Microscopic Examination of the Brain:

Lenticular Nucleus: Edema; degeneration of small arterioles; multiple polymorphs in a large vein; pyknosis; glia cell-infiltration around degenerated vessels in a dense meshwork of fibrillae; fibrinous thrombi; arteries slightly thickened.

Internal Capsule: The same.

Cortex: Punctiform hemorrhages in the leptomeninges; arteriosclerosis.

Remarks: Was overcome by gas; pulmotor used at house, then taken to the hospital, one block away, where patient died thirty minutes later.

Case 29.—Man, aged 65, found dead.

Anatomic Diagnosis: Crimson colored emaciation; general senility; barrel-shaped chest, asymmetric; distended superficial veins; opacity of the cornea of the right eye.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; moderate internal hydrocephalus; sclerosis of the cerebral arteries.

Microscopic Examination of the Brain:

Lenticular Nucleus: Marked hyperemia; capillary hemorrhages; marked periarteritis; hematosiderin in the vessel walls and perivascular tissue; multiple spherical bodies surrounding the degenerated capillaries; marked edema; large fibrinous thrombi; sclerosis of the arteries.

Corona Radiata: Arteriosclerosis; edema; hyperemia; degenerated capillaries; endothelial leukocytes filled with hematosiderin.

Remarks: Found dead in his room (a card found in his home stated that he was old, he had no money and no friends, and he wanted to die).

Case 30.—Man, aged 65, found dead.

Anatomic Diagnosis: Cherry-red blood; marked edema of the lungs; marked hyperemia of the lining of the trachea and larger bronchi; catarrhal conjunctivitis; first degree burn of the right buttock; petechial hemorrhages in the lining of the stomach; obesity; marked subepicardial infiltration with fat; disseminated atrophy of the cerebral cortex; lessened yellow material in the suprarenal cortices; syphilitic(?) arteriosclerosis; marked syphilitic(?) scars on the legs; chronic diffuse nephritis; secondarily contracted kidneys; large biliary and periaortic lymph glands; distended urinary bladder; fibrous external pacymeningitis; fibrous pleuritis; fibrous capsular perisplenitis; slight sclerosis of the anterior mitral leaflet.

Macroscopic Examination of the Brain: Disseminated atrophy of the cerebral cortex; partial anemia and hyperemia of the leptomeninges; hyperemia of the brain; punctiform hemorrhages(?) in the brain stem; perivascular, fibrous leptomeningitis; internal hydrocephalus; slight sclerosis of the cerebral arteries; pinkish coloration of the brain substance; marked anomaly in size between the vertebral arteries.

Microscopic Examination of the Brain:

Leptomeninges and Cortex: Edema; partial hyperemia and partial anemia.

Lenticular Nucleus and Internal Capsule: Ischemic necrosis; edema; degeneration of the vessel walls; spherical bodies.

Remarks: Found dead in closed room with gas jet open; had expressed wish to die.

Case 31.—Man, aged 40.

Anatomic Diagnosis: Carbonylemia; extensive first and second degree burns of the face, trunk and extremities; slight edema of the glottis; marked hyperemia of the respiratory passages; marked edema of the lungs; alcoholic odor of the abdominal cavity; chronic follicular tonsillitis; slight hyperplasia of the spleen; slight senile sclerosis of the aorta; large fatty liver.

Macroscopic Examination of the Brain: Hyperemia of the brain and leptomeninges; edema of the brain; internal hydrocephalus; pink discoloration of the white substance; slight bilateral softening of the lenticular nucleus; cystic degeneration of the choroid plexus; sclerosis of the cerebral arteries.

Microscopic Examination of the Brain: Softening grossly seen.

Remarks: Found dead in bed on a second floor; there was a fire on the first floor.

Case 32.—Man, aged 27, survived a few hours.

Anatomic Diagnosis: Extensive, spontaneous, submucous hemorrhages in the small bowels; petechial hemorrhages in the lining of the stomach, urinary bladder, cecum, small bowel and pericardium; hyperplasia of the spleen; fatty changes in the aorta and iliac arteries; slightly fatty liver; atrophy of the left testicle; papular eruption of the right thigh; fibrous adhesions between gallbladder and great omentum.

Macroscopic Examination of the Brain: Atrophy of the cortex; marked hyperemia of the brain and leptomeninges; multiple punctiform hemorrhages in the brain and leptomeninges; marked internal and external hydrocephalus; slight perivascular fibrous leptomeningitis; circumscribed, bilateral softening of the corpus striatum; bright pink coloration of the brain substance; marked sclerosis of the cerebral arteries; anomaly in size of the left vertebral artery.

Microscopic Examination of the Brain:

Lenticular Nucleus and Internal Capsule: Necrosis; degeneration of the vessel walls; hyalin thrombi; arteriosclerosis; edema; numerous spherical bodies.

Cortex and Leptomeninges: The same, plus fibrous thickening of the meninges.

Remarks: Found unconscious in his room and died on the way to the hospital; the father had been told by two attending physicians that his son was suffering from gas poisoning.

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